STUDIES ON THE METAL UPTAKE POTENTIAL, MECHANISM AND APPLICATIONS OF BIOSORBENT *Ganoderma lucidum*

A Thesis Submitted
in Partial Fulfilment of the Requirements
for the Degree of

DOCTOR OF PHILOSOPHY

by

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to the

DEPARTMENT OF CIVIL ENGINEERING
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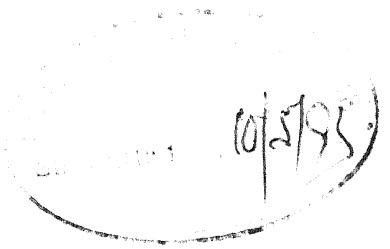
MAY, 1993

DEDICATED TO

SUDHA AKKA

just to reassure that her hardwork and sacrifices
which went into the making of good research
will not go unnoticed



CERTIFICATE

It is certified that the work contained in the thesis entitled "Studies on the Metal Uptake Potential, Mechanism and Applications of Biosorbent *Ganoderma lucidum*", by Mr. Muraleedharan T.R., has been carried out under my supervision and that this work has not been submitted elsewhere for a degree.

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May, 1993

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MURACY

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a, a′, b, b′	Constants in BDST Model
b	Langmuir's Constant
C_A	Concentration of Adsorbate in Solution at Any Time, ML^{-3}
C_B	Concentration of Adsorbate on the Sorbent at Any Time
C_{Ao}	Initial Concentration of Adsorbate in Solution, ML^{-3}
C_{Bo}	Initial Concentration of Adsorbate on the Sorbent, ML^{-3}
C_{Be}	Equilibrium Concentration of Adsorbate on the Sorbent
C_o, C_1	Concentration of Metal in the Influent to the Column, ML^{-3}
C_b, C_f	Desired Concentration of Adsorbate at Breakthrough, ML^{-3}
C_e	Concentration of Adsorbate in Liquid Phase at Equilibrium, ML^{-3}
C^l	Concentration of Adsorbate in Liquid, ML^{-3}
C^f	Concentration of Adsorbate in Film, ML^{-3}
C^{l*}	Concentration of Adsorbate in Solution Adjoining Adsorbent, ML^{-3}
C^s	Concentration of Adsorbate in Solid
C_e^s	Equilibrium Concentration of Adsorbate in Solid Phase
C_e^l	Equilibrium Concentration of Adsorbate in Liquid Phase, ML^{-3}
C_t	Concentration of Adsorbate in Solution at Any Time, ML^{-3}
D^l	Diffusion Coefficient in the Liquid, M^2T^{-1}
K_c	Equilibrium Constant, $L^3 M^{-1}$
k′	Overall Reaction Rate Constant
K_c	Conditional Stability Constant
K	Rate Constant of Adsorption in Column
k_1, k_2	Proportionality Constants
K_{tot}	Overall Stability Constant
K_{sp}	Solubility product
N_o	Adsorptive Capacity of Sorbent
P	Rate of Permeation, $M^2L^{-4}T^{-1}$
Q_{max}	Maximum Number of Adsorption Sites Available on the Sorbent
q_e	Equilibrium Coverage of adsorbate over the adsorbent
Q_∞	Total amount of Adsorbate at equilibrium on adsorbent

Q	Concentration of Adsorbate per particle
q	No sites on the adsorbent occupied by adsorbate
$U(t)$	Fractional Attainment of Equilibrium
r_o	Radius of Adsorbent Particle, L
r'_o	Radius of Liquid Film Around Adsorbent, L
R	Reaction Rate Constant
T	Service Time of Column, T
t	Time, T
V, V'	Linear Flow Velocity of Feed, LT^{-1}
X	Weight of Biosorbent, M
X	Depth of Column, L
X_A	Fractional Binding of Metal to Sorbent at Any Time
X_{Ae}	Fractional Conversion of Adsorbate at Equilibrium
Y	Yield Coefficient
θ	Fractional attainment of equilibrium
Δr_o	Thickness of Liquid Film Around Adsorbent, L
$\varkappa$	Distribution Coefficient

SYNOPSIS

Studies on the Metal Uptake Potential, Mechanism and Applications of Biosorbent *Ganoderma lucidum.*

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Biosorption, the process in which microorganisms (dead or alive) or their derivatives are employed for the removal and recovery of toxic metals, is one of the rapidly emerging pollution control technologies. Within a brief period, the idea of biosorption has evolved from a mere concept to a practicable technology with the emergence of many patented microbial based sorbents developed to field scale reactors.

Though there are several reports dealing with the potential of laboratory grown microorganisms (algae, bacteria and fungi), fungal byproducts of industrial fermentation, marine algae and activated sludge for heavy metal uptake, little exploration work has been carried out on the possibility of employing naturally available macro fungi. These are not only widely available but also appear to possess properties suitable for continuous flow operation. An exhaustive investigation to utilize the biomass as biosorbents for metal removal from aqueous phase is hence warranted.

A set of nine commonly available species of wood rotting mushrooms selected for this investigation were identified at Royal Botanical Gardens (Kew, ENGLAND). These were sun dried and powdered to a size range of 600–1200 μm. The preliminary screening of fungi was carried out on the basis of quantitative determination of of maximum metal (copper (II)) uptake potential (Q_{max}) using Langmuir isotherm. The maximum metal uptake varied from 0.048 mmol/g for *Trametes lactenia* to 0.383 mmol/g for *Ganoderma lucidum.* Since the latter exhibited an excellent metal uptake

compared to other species, *Ganoderma lucidum* was selected for further studies.

A thorough scan of literature on biosorption revealed the deployment of wide variety of chemical treatment to microbes to improve their engineering properties. To investigate the effect of this, the biosorbent *G. lucidum* was subjected to pretreatment using a number of chemicals like methanol and chloroform, cold alkali and hot alkali sequentially to obtain derivatives mainly devoid of lipids, proteins etc. The biosorbent was also subjected to formaldehyde treatment both in H_2SO_4 and HNO_3 background. The resultant derivatives were compared based upon a broad spectrum of physico-chemical properties desirable for a potential biosorbent (thermal stability, physical strength, leaching characteristics, chemical stability, head loss, filterability etc.) in addition to their metal uptake capacity. The results indicated that the biosorbent *G. lucidum* does not require any elaborate chemical treatment to behave as a good and potential biosorbent thus eliminating the cost of pretreatment. Further investigation was then carried out with *G. lucidum* in its native form without any chemical treatment.

Mechanism studies employing transmission electron microscope provided evidence to the fact that the metal was accumulated in the cell wall rather than in the cytoplasm. Energy Dispersion X-Ray Analysis (EDAX) indicated release of calcium ions into the aqueous medium from the biosorbent during the uptake of copper ions. The derivatives resulted after sequential elution of cellular components were subjected to spectroscopic studies (Infra red, and Electron Paramagnetic Resonance (EPR)). The results revealed that copper was coordinated to the structural proteins and polysaccharides. The EPR spectra indicated that

copper was coordinated in a square planar geometry with four surrounding atoms. Comparison with similar spectra reported in literature indicated that the coordinating ions are nitrogen and oxygen. The EPR spectra along with EDAX spectra appear to indicate that the donor ligand of the biosorbent to be rich in oxygen atoms and hence a preferential uptake of oxygen seeking elements can be expected. This hypothesis was verified from the results of preferential uptake of lanthanum (an oxygen seeking element) from an equimolar mixture copper and lanthanum. Other metals (Cr, Mn, Co, Ni, Zn, Cd, Hg) were also taken up to varying degrees depending upon the availability of preferred donor sites.

The potential application of this biosorbent for pollution control in the rare earths industry was investigated since rare earths elements are oxygen seeking. Further, rare earths also belong to the 'metal' category and very little attention has been focused on their control though they have deleterious effects on environment. Being of strategic importance, their recovery and reuse from process effluents will facilitate optimal resource utilization. The practical reason for selecting the effluent from this industry as model case for the application of biosorption was that rare earth processing extensively employs ion-exchange resins and hence the resistance to the introduction of a similar technology for pollution control is expected to be minimum.

The potential of employing the biosorbent *G. lucidum* for removal of rare earths was assessed by conducting the equilibrium sorption experiments using rare earth chloride (RCl_3). Langmuir plot of adsorption data gave maximum uptake (Q_{max}) of around 0.30 mg/g for all rare earth elements. Kinetics data also presented a very favorable rate of uptake with more than 50 % of uptake in 3 minutes and most of the

possible removal taking place in 30 minutes. In continuation with batch studies, column reactors were also run employing a set of 3 packed bed reactors of diameter 50 mm and height varying from 300 to 1200 mm using an influent containing 250 mg/l of RCl_3 at a flow rate of 1000 $L/m^2/h$. The column runs were terminated at breakthrough point. The adsorbed rare earth elements could be desorbed by employing 0.1 N HCl and a concentration factor of 40 was achieved. The simulated monazite processing industrial effluent consisting of high doses of fluorides and phosphates, traces of heavy metals (lead and zinc) in addition to thorium and rare earths could also be effectively treated by fixed bed reactor employing biosorbent *G. lucidum*. The results of the column studies indicated that target elements were brought below levels of detectability.

Separation of rare earth elements is a difficult task and has been traditionally achieved by employing ion-exchange resins. In the present study, separation of adjacent rare earth elements praseodymium and neodymium was attempted using biosorbent *G. lucidum* in a long column. The column was loaded with a 50:50 mixture of Pr:Nd at a flow rate of 1000 $L/m^2/h$ and elution was carried out by 5% citric acid (pH 2.54), the flow rate being maintained at 180 cm/h. The results revealed that the concentration of Pr could be enhanced many times with Nd concentration remaining constant.

The present investigation thus resulted in the development of a biosorbent of macrofungus origin, assessment of its potential for removal of a broad spectrum of metals and elucidation of metal uptake mechanism. The study also resulted in utilisation of this process for resource recovery and pollution control in monazite processing industry.

1. INTRODUCTION

Metallic pollutants, because of their ubiquity and non-degradability, continue to be a major concern to environmental engineers. The expanding toxicological database on metals combined with the availability of sophisticated instrumentation, has led to the establishment of stringent standards for disposal of metal bearing industrial effluents. As a result, the existing treatment technologies are becoming obsolete from both efficiency and economy point of view and consequently many treatment alternatives are being developed and evaluated over the past decade to meet the ever increasing demand to satisfy effluent standards.

Biosorption, in which microbes are directed to accumulate metals from the aqueous solutions has emerged as a promising alternative technology in the last decade. From just a novel idea in early eighties, biosorption have evolved into a commercial process in a short duration. The undeniable fact that biosorption has become popular is exemplified by the fact that the Society of Chemical Industries (UK) considered it appropriate to organize a specialized conference for a realistic assessment of the commercial feasibility of this process (Eccles, 1990).

Though the potential of microbes to take up metal from their surrounding aqueous medium far in excess of their metabolic requirement was known for a long time, the creative direction of this potential for pollution control is rather new (Muzzareli and others, 1980; Tsezos and Volesky, 1981). The enhanced research effort in this area is also a result of the revived global interest in biotechnology (Anonymous, 1986).

Biosorption studies classically involve the screening of a group of microbial biomass for their metal uptake, followed by the detailed evaluation of promising biosorbents. After this step, the engineering properties of the biosorbents are evaluated and the biosorbents chemically modified, if necessary, before conducting bench scale studies for scale up purposes. The organisms studied thus far encompass bacteria, algae, fungi, and heterogeneous microbial system like activated sludge. Metal uptake studies have been conducted with both viable and non viable species of microbes. The commercial developments till now, however, have utilized only non-viable species due to the the ease and simplicity of process maintenance.

In spite of the concerted effort from many disciplines, the mechanism of biosorption has not yet been elucidated comprehensively. Metal uptake by viable organisms could be metabolically mediated transport in addition to chemical coordination to cell wall. In the case of non-viable cells the later is the only possible mode of metal uptake. Previous studies have identified the structural proteins and carbohydrates as the coordinating sites though the possible involvement of other cellular components were not ruled out. It is imperative to state that a thorough understanding of the mechanism is expected to greatly aid the reactor design and operation

The vast biological diversity available in the tropical forests represent one of the major avenues for new developments in biotechnology (Newmark, 1983). While laboratory grown cultures of microbes and biomasses which are byproducts of industrial fermentation processes have been evaluated extensively, no concerted effort has been made so far to explore the possibility of utilizing

naturally available biomass saphrophytic macrofungi abundantly available in tropical forests.

The objectives of the present investigation include screening of the macrofungi for their metal uptake, imparting chemical treatment to the selected biosorbents to improve their properties for use in continuous flow reactors, bench scale experimentation for scale up purposes and the delineation of the mechanism of biosorption. Besides copper as a model metal, an array of heavy metals, rare earth elements and monazite processing effluents were used in the studies.

2. LITERATURE REVIEW

2.1 SCOPE

The detrimental effects of metals on environment and the possible ways and means of scavenging them have been subject of active research in the last three decades. Increased awareness consequent to publicized episodes such as Minamata, availability of sophisticated instrumentation which enabled the accurate determination of metal concentration to parts per billion levels, and availability of toxicological data which suggested that most metals have deleterious effect on health at higher concentration have all contributed to the enhanced research activity in this area.

A number of techniques have been proposed and utilized for the scavenging and control of metals from metal bearing effluents. One of the new generation technology for metal pollution control is biosorption, which is the subject matter of the present work.

The literature review cover the following aspects:

A. Metals in aqueous environment and their removal from the effluents
   1. Biological importance of metals
   2. Deleterious effects of metals on health
   3. Sources of metallic pollutants
   4. Existing control technologies for metals

B. Biosorption as an alternative metal removal technology
   1. History of metal-microbe interaction
   2. Use of live microbes for metal uptake
   3. Use of non-viable microbes for metal uptake
   4. Mechanism of metal uptake by biosorbents

C. Rare Earths as resources and pollutants

1. Rare earths: sources and uses

2. Processing and sources of effluents

3. Health effects and pollution control

2.2 ENVIRONMENTAL EFFECTS OF METALS

2.2.1 Metals and Their Biological Significance

The term 'metal' designates an element in the periodic table that is a good conductor of electricity and whose electrical resistance is directly proportional to the absolute temperature. In addition to this typical character, they also share many other physical characters such as high thermal conductivity, high density, malleability and ductility. Studies in the field of bioinorganic chemistry have indicated that trace quantities of many of these elements exert a positive or negative influence on the biological organisms and hence the biosphere. The occurrence of a few widely published environmental catastrophes like the Minamata bay episode (Kutsuna, 1968) coupled with the development of sophisticated analytical techniques in the recent period for the measurement of microconcentration of metal ions, have aided scientists to appreciate the nature and extent of the impact of these elements on the biosphere.

Metals, though ubiquitous in the environment, is unique as compared to other pollutants, as they can neither be created nor destroyed. There has been a well established bio-geochemical cycle for metals even before man appeared in the scene. However, in the period since industrial revolution, man has greatly accelerated the rate at which the material can be recovered from nature which has

disturbed this cycle. Activities starting from mining to metal processing to manufacturing to metal finishing bring into the environment metals at a rate which has been never encountered by nature in the geologic past and as a result, the conventional regulatory mechanisms, be it biological or physico-chemical, fail to control levels of metals to desirable limits in the environment. It is this enhanced input of metals in concentrations and combinations unparalleled in mankind's history, which is causing concern and not the mere presence of the metals which were always present in nature and the biota have adapted to utilise beneficially in their metabolic processes.

It is also to be understood that metals are one of the major raw materials of our modern way of life, and progress in metallurgy has been synonymous with progress of civilization (Iron and Copper Ages) that any attempt to curtail their utilization is going to be counter-productive. The task of an environmental engineer hence is to regulate their influx into environment in biologically assimilable forms within acceptable levels.

Metals are unique as a group of pollutants also in the sense that many of these metals considered harmful at higher levels are essential for the growth of biota in trace concentrations. Many biomolecules like enzymes depends on the metal components in their molecular structure to carry out their functions. Of more than twenty elements which have so far been identified as essential for microbial life about two thirds belong to the category of metals, though not all these metals are essential for the growth and cell division of every microbial species. A comprehensive compilation of essential metals

and their metabolic functions is presented in Figure 2.1. An overabundance of any of these elements can cause build up to intracellularly toxic levels, which result in chronic and acute troubles. Typical dose response curves for essential and non essential metals are presented in Figure 2.2.

2.2.2 Toxicological Details of Metals

Metals are probably the oldest toxins known to humans. Hypocrates is credited in 370 BC with the first description of abdominal colic in men who extracted metals. Arsenic and mercury are cited by Theophrastus of Erbus (375 BC) and Pliny the elder (AD 23-79) as disease causing agents (Goyer, 1986). Many of the metals of toxicological concern today are, however, known only recently to humans, since their supply was confined to certain geographical locations and usage limited to those places.

Viewed from environmental health point of view, metals may be classified according to three criteria

a. Non critical

b. Toxic but less available to biota as they are insoluble or rare

c. Very toxic and relatively accessible

A detailed classification of the elements according to the above criteria is given in Table 2.1.

The manifestation of the health effects of heavy metals is not unique. It could vary from subclinical forms not directly attributable to heavy metal pollution like lowering of IQ of children exposed to chronic lead poisoning to bloody diarrhoea and suppression of urine formation from ingestion of corrosive mercury. Carcinogenecity is an important characteristic of many heavy metals

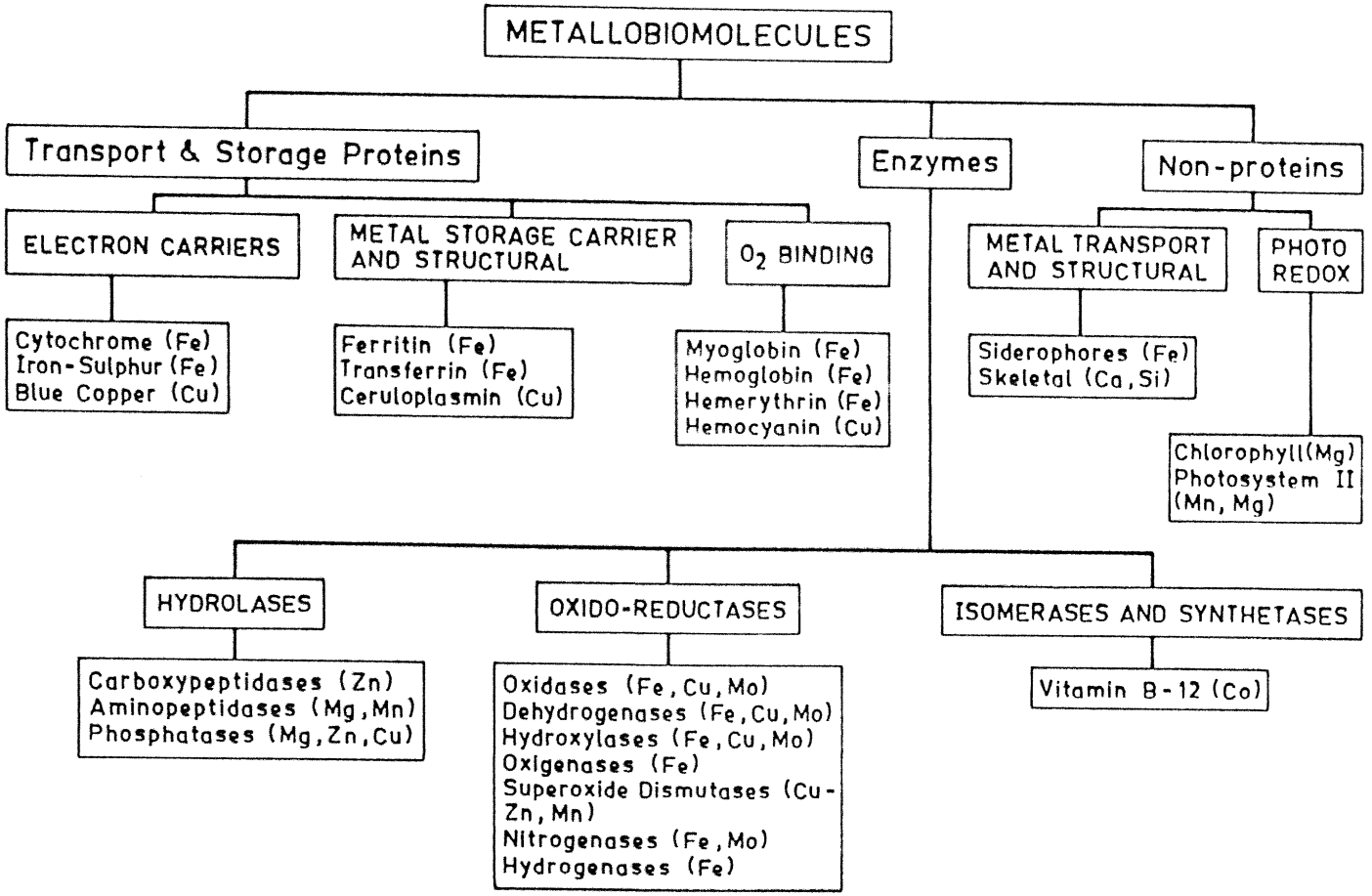


Fig. 2.1. Important Metals with Biological Functions.
(Ref. Ibers and Holmes, 1980)

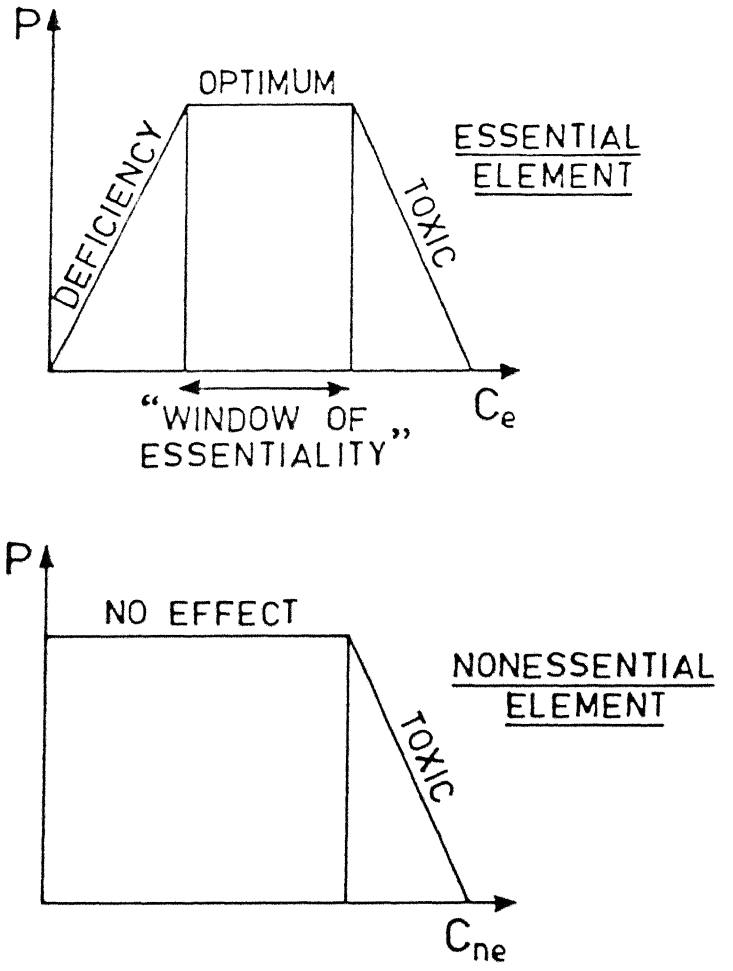


Fig. 2.2. Relationships Between Performance (P) (Growth, Fecundity, Survival) and Concentration of an Essential (C_e) or Non-Essential Element (C_{ne}). (Ref. Hopkins, 1989)

Table 2.1 Classification of Elements Based on Toxicity and Availability*

Criteria	Elements
Non-Critical	Na, K, Mg, Ca, H, O, N, C, P, Fe,S, Cl, Br, F, Li, Rb, Sr, Al, Si
Toxic but very rare/ very insoluble	Ti, Hf, Zr, W, Nb, Ta, Re, Ga, Lanthanides, Os, Rh, Ir, Ru, Ba, Actinides
Toxic and relatively accessible	Be, Co, Ni, Cu, Zn, Sn, As, Se, Te, Pd, Ag, Cd, Pt, Au, Hg, Tl, Pb, Sb, Bi

* Forstner and Wittmann, 1981

and in spite of the long history of human exposure to metals and understanding their toxicity potential, data regarding the potential carcinogenecity is now only evolving. This aspect is covered in detail in following pages.

Most metals affect more than one organ and the targets of toxicity are specific biochemical processes and/or membranes of cells. The toxicity generally is a result of an interaction between the free metal ion and the susceptible target. In many instances, toxicity is a case of mistaken identity of the toxic element as an essential element, for example, lead for calcium in the central nervous system. Cells and components such as gastro-intestinal, liver or renal tubular cells, which are involved in transport of essential metals, are particularly susceptible to toxicity. Table 2.2(a) to (c) is a compilation of the toxic effects of some metals

Many exogenous factors like age, diet, and interactions and concurrent exposure with other toxic metals also influence toxicity of metals for a particular subject . Young children are more susceptible to toxicity from exposure than the adults, primarily because they consume more food per body weight than adults. Rapid cell division in children represent opportunities for genotoxic effects. A diet rich in vitamin C or D tend to reduce the toxicity of lead and cadmium probably because of increase in absorption of ferrous ions. An inverse correlation between protein content of diet and lead and cadmium toxicity has also been reported (Nordberg and others, 1981).

2.2.2.1 Speciation of Metals and Effect on Toxicity

Other than the concentration of metal as such, its speciation also affects the intestinal absorption, body

Table 2.2 Major Toxic Metals With Multiple Effects*

(a) Non-Essential Metals

Metal	Acute Exposure	Chronic Exposure
Arsenic	Sensory loss Cardiovascular Effects	Liver injury Blackfoot Disease
Beryllium	Pneumonitis	Beylliosis
Cadmium	Abdominal pain	Chronic bronchitis Kidney Damage
Chromium	Renal tubular necrosis	Ulceration of Nasal septum
Lead	Peripheral neuropathy	Renal effects
Mercury	Corrosive Bronchitis Bloody diarrhea	Nervous system damage, Renal damage Pink disease
Antimony	Rhinitis	Pharyngitis
Barium	Muscular paralysis	Baritosis
Magnesium	Renal impairment	Conjunctivitis
Silver	Arteriosclerosis	Argyria
Tellurium	Cyanosis	Necrosis of liver
Thallium	Paralysis	Hair loss
Tin	Cerebral edema	Visual defects

(b) Essential Metals

Metal	Acute Exposure	Chronic Exposure
Cobalt	Polycythemia	Goiter
Copper	Coma	Wilsons Disease
Iron	Hepatic cirrhosis	Transfusional sideriosis
Manganese	Mn pneumonitis	Manganism
Molybdenum	Degeneration of liver	Teart
Selenium	Nervous system failure	Garlic breath
Zinc	Diarrhea	Testicular tumor

(c) Metals with Medicinal Use

Metal	Medicinal Use	Chronic Exposure	Acute Exposure
Aluminium	Dialysis Fluid	Osteomalacia	Pulmonary Fibrosis
Bismuth	Antacid	Renal failure	Nervous system effects
Gallium	Radiological tracer		Bone marrow depression
Gold	Treatment of rhumatism		Dermatitis
Lithium	De-depressant	Coma	Nephrotoxicity

* Goyer, 1986.

distribution and ultimately its toxicity (Nielson and Anderson, 1970). Phosphates generally form less soluble salts of metals than other anions, hence rendering the metal relatively less toxic. Alkyl compounds such as tetraethyl lead and methyl mercury are lipid soluble hence easily migrate across the cell membrane leading to low tolerance levels (Doi and Ui, 1975). However, in general, free metal ions are more active and hence more toxic (Hart and Davies, 1978). Species of metals which are bound either to ligands or particulates are less available thus causing a dampening effect on its toxicity .

2.2.2.2 Immunological Response to Metal Toxicity

The immune status of an individual becomes an additional toxicologic variable for metals that produce hypersensitivity reactions. Such metals include mercury, gold, platinum, beryllium and nickel. In an immediate hypersensitivity reaction, the antibody, IgE, react with the the antigen of the surface mast cell releasing vasoreactive amines. Clinical reactions include conjunctivitis, asthma, urticaria or systemic anaphylaxis. Cutaneous, mucosal and bronchial reactions to platinum have been attributed to this type of hypersensitivity reactions. Cytotoxic hypersensitivity is the result of a compliment fixing reaction of the IgG immunoglobulin with antigen or hapten bound to the cell surface. The thrombocytopenia sometimes occurring with organic gold salts may be brought about in this manner. Immune complex hypersensitivity occurs when soluble immune complex deposits (antigen, antibody and complement) within tissues producing an acute inflammatory reaction. Immune complexes which are produced are generally accumulated on the epithelial surface of glomerular basement membrane resulting in

proteinurea and occur following exposure to mercury vapour or gold therapy. Cell mediated hypersensitivity, also known as the delayed hypersensitivity reaction mediated by the thymus dependent lymphocytes usually occurs 24 to 48 hours after exposure.

2.2.2.3 Metals as Carcinogens

The relation between cancer and metal ions is attracting more and more research. Epidemiological correlation between different cancers and the distribution of some trace elements in the ambient environment (soil, water and air) have been suggested. A number of metal ions are confirmed carcinogens while few more are on the suspected list. Table 2.3(a) and (b) is a compilation of the carcinogenecity of various metals.

2.3. ANTHROPOGENIC SOURCES OF METALS

Metallic pollutants are disposed into the aquatic environment from many sources, the most important of these sources being the metal processing and finishing industries. However a number of other industries also employ metals in their processes and let out effluents with toxic levels of these elements. Table 2.4 gives a comprehensive list of industries using metals and the effluents from these contain high concentrations of metals.

2.4 HEAVY METAL MANAGEMENT STRATEGIES

A number of techniques have been developed for the treatment of metals from industrial effluents. They can broadly be classified into destructive and recovery methods. Destructive methods are not really destructive as the name implies, but remove the metal from the effluent in a form in which it is generally disposed off and not reused, thus for all practical purposes this fraction of metal is

Table 2.3 Genetic Toxicity of Metal Carcinogens*

(a) Confirmed Carcinogens

Metal	Carcinogenecity	Mutagenecity	Chromosome Effects
As(V)	Human		+
As(III)	Human	+	+
Be (II)	Animal	+	+
Cd(II)	Human & Animal	+	+
Co(II)	Animal	+	-
Cr(VI)	Human & Animal	+	+
Fe(II)	Animal	+	+
Ni	Human & Animal	+	-
Pb	Animal	+	-
Zn	Animal	+	+

(b) Suspected Carcinogens

Metal	Carcinogenecity	Mutagenecity	Chromosome Effects
Ag (I)	Animal	+	-
Al(III)	-		+
Cu(II)	Animal	+	+
Hg	-	-	+
Mn	Animal	+	+
Mo(VI)	-	+	+
Pt(II)	Anti tumor	+	+
Sb(V)	-		+
Se(VI)	Anti carcinogen	+	-
Te(IV)	-		+

* Flessel and others, 1980.

Table 2.4 Heavy Metals Employed in Major Industries*

Sl. No.	Name of Industries	Cd	Cr	Cu	Fe	Hg	Mn	Pb	Ni	Sn	Zn
1.	Pulp, papermills, paper, board, building paper board mills		X	X		X		X	X		X
2.	Organic chemicals, petro-chemicals	X	X		X	X		X		X	X
3.	Alkalis, chlorine, inorg-anic chemicals	X	X		X	X		X		X	X
4.	Fertilizers	X	X	X	X	X	X	X	X		X
5.	Petroleum refining	X	X	X	X			X	X		X
6.	Basic steel works, foundaries	X	X	X	X	X		X	X	X	X
7.	Basic nonferrous metal works, foundaries	X	X	X		X		X			X
8.	Motor vehicle, aircraft plating finishing	X	X	X		X		X			
9.	Flat glass, cement, asbestors production etc.		X								
10.	Textile mill products		X								
11.	Leather, tanning, finishing		X								
12.	Steam generation power plants		X								

* Forstner and Wittmann, 1981.

lost. In the case of recovery methods, the metal is removed from the effluents and is recycled as a resource.

2.4.1 Destructive Methods:

2.4.1.1 Alkaline Precipitation:

The metal from industrial effluent is removed as metal hydroxide precipitate in the alkaline precipitation methods. The pH of the metal bearing solution is raised to a value where the solubility of the metal hydroxide is minimal. The resulting precipitate is separated using solid liquid separation process followed by its disposal.

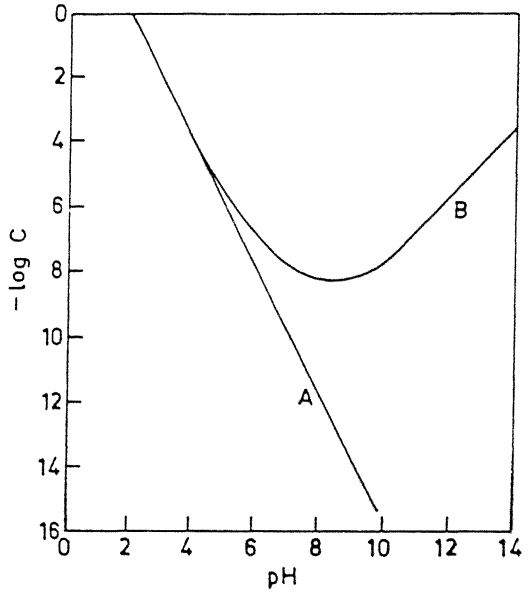
The simple chemical reaction of chemical precipitation may be written as

$$M^{n+} + n\ (OH)^{-} \longrightarrow M\ (OH)_n \qquad 2.1$$

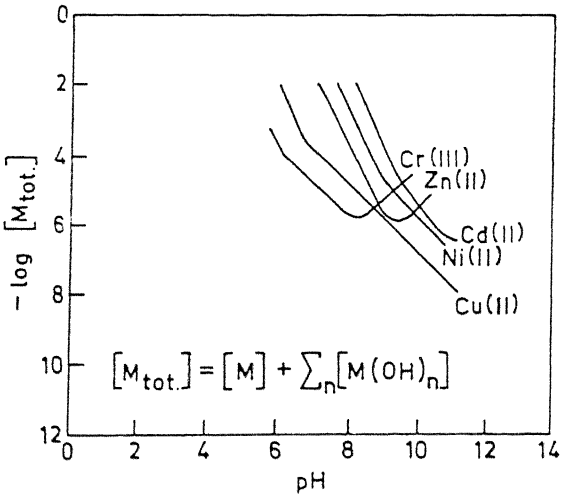
the solubility product for this can be written as

$$K_{sp} = \frac{[M][OH]^n}{[M(OH)_n]} \qquad 2.2$$

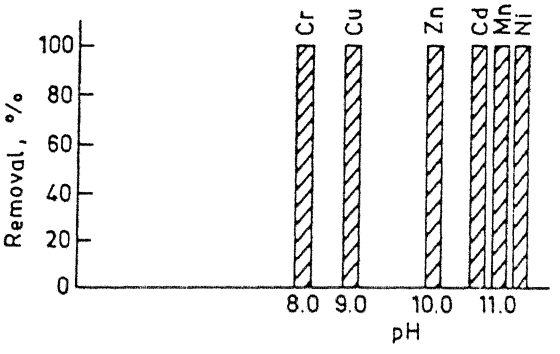
In the reallife situation, it is not possible to obtain the soluble metal concentration as predicted by the solubility product equation. This is because metals form soluble metal complexes with the complexing agent. Each of these complexes will be in equilibrium with the solid phase and the resultant solubility will be a function of the complex equilibrium thus formed. Figure 2.3.(a) illustrates the typical theoretical and practical solubility curves of metals. The solubility behaviour of some metals is graphically presented in Figure 2.3 (b). The pH for least solubility for different metals is given in Figure 2.3 (c).



(a) Theoretical Solubility of Hypothetical Metal Hydroxide $M(OH)_2$, (A) with Complexation and (B) without Complexation.



(b) The Solubility of Pure Metal Hydroxides as a Function of pH.



(c) The pH of Optimum Percent Removal by Lime Neutralization for Selected Metals.

Fig. 2.3. Hydroxide Precipitation as a Method of Heavy Metal Pollution Control. (Ref. Patterson and Minear, 1975)

2.4.1.2 Sulfide Precipitation

Metallic sulfides have a lower solubility than their hydroxides and hence sulfide precipitation has been employed as an alternative when standards cannot be met by the hydroxide precipitation method. Table 2.5 shows the solubility product data of metal sulfides vis-a-vis metal hydroxides.

One major drawback of the sulfide precipitation is that most of the metal sulfides are readily soluble in acidic pH and hence after disposing off the sulfide sludge the possibility of leaching of metal is very high. This necessitates disposal of sludge in chemically secured landfills and perennial ground water monitoring to check for pollution.

2.4.2 Recovery Methods

2.4.2.1 Electroplating

Electrolytic techniques are effective to plate out metals, oxidise cyanide or reduce chromium from wastewaters. Electricity is the only operating cost and no chemicals are required. A typical arrangement employs an electrolytic cell with a cathode bed of thin metallic particles, a graphite particle bed anode and a cellophane separator. Direct current of low voltage is applied and best removals (90-95%) are obtained when NaCl is added as a supporting electrolyte with recirculation of the anode bed effluent. However, for dilute solutions such as less than 100 ppm of pollutant metal level, the electrical resistance of solutions is high and cost of electricity becomes prohibitive.

Table 2.5. Solubility Products of Metal Hydroxydes and Sulfides*

Hydroxides	-Log K_{sp}	Sulfides	-Log K_{sp}
$Mn(OH)_2$ (Cryst)	12.7	MnS	12.6
$Cd(OH)_2$	14.4	CdS	27.8
$Fe(OH)_2$	15.1	FeS	17.2
$PbO+H_2O$	15.3	PbS	27.5
$Co(OH)_2$ (Pink)	15.7	CoS	24.7
$ZnO+H_2O$ (aged)	16.8	ZnS	21.6
$Ni(OH)_2$	17.2	NiS	25.7
$CuO+H_2O$ (Tenorite)	20.5	Cu_2S	48.0
$HgO+H_2O$	(25.4)	HgS	52.4

* Sillen and Martell, 1964

2.4.2.2 Ion Exchange Technology

Ion exchange methods are employed for recovery of precious metal from effluents having very low metal concentrations. The most essential component of this process is an ion exchange resin, which can exchange H^+ or Na^+ ions in lieu of the metal ions to be removed from the effluent. The effluent and the resin is generally contacted in a packed bed reactor with the toxic wastewater being pumped from the top. The transfer of the toxic metal from liquid phase to solid phase is achieved within the bed which get exhausted after some time. Once the bed is no longer able to provide effluents of required quality, it is regenerated with acid or brine solution and can be reused.

Resins used for ion exchange can be natural zeolites or synthetic polymeric network with charged functional group which attract oppositely charged ionic species (Singer, 1974). Resins exhibit a relative affinity to all ionic species of opposite charge depending upon their ionic charge, ionic radius, concentration in solution and the nature of the functional groups. Copper, lead, mercury and nickel have been successfully treated by cation exchange process (Schore, 1972; Cheremisinoff and Habib, 1972; Patterson and Minear, 1975).

The major draw back of ion exchange process is their prohibitive cost. Secondly, presence of any ligand which complex the metal (like cyanide, EDTA etc.) will drastically reduce the efficiency. Other impurities like organic material, oil, fat, dust etc., causes the fouling of the ion exchange resins preventing the attainment of

maximum capacity and hence the wastewater requires adequate pretreatment (Kuyucak, 1990).

2.4.2.3 Membrane Separation Processes.

Membrane processes such as electrodialysis, dialysis, reverse osmosis and ultra filtration have been suggested and employed as alternatives for heavy metal control (Huack and Saurirajan, 1972). A membrane is a phase which act as a barrier between other phases and achieve the separation of metals from effluent by either selective retention of the metal or allowing migration of only water whereby concentrating the metal. Membrane materials for reverse osmosis are fairly limited and most of the work has been done with cellulose acetate (Hall and others, 1979). Cellulose acetate has a workable range only in pH 4-8 and a temperature range 20-30 ^{o}C limiting the utility of this process only to selected industries.

2.4.2.4 Adsorption

Adsorption on a number of solid surfaces have been gainfully employed for the removal of metals from industrial effluents (Faust and Aly, 1987). The adsorbents which have been tried include commercial activated carbon, activated alumina, iron coated sand, iron ore and a variety of low cost adsorbents. Though activated carbon has exhibited its potential to scavenge a number of metals, their uptake capacity is generally small and as such adsorption has only been attempted as a polishing unit for a conventional treatment unit than being an effective alternative. Investigations with other adsorbents are still in the laboratory scale and no field data is available regarding the applicability and drawbacks of the process.

However, judging from the low uptake capacity being reported for these adsorbents, they are also likely to be employed only as polishing units.

A comparative evaluation of various existing metal bearing effluent treatment options is presented in Table 2.6.

2.5 BIOSORPTION: AN ALTERNATIVE METAL RECOVERY TECHNOLOGY

One of the emerging technologies which utilises the potential of biological organisms to take up substantial quantities of metals from their ambient environment, has shown good promises. This concept, termed biosorption, is broadly defined as "processes whereby microorganisms, alive or dead, or their derivatives, are employed for the sequestration of heavy metals from the environment".

The capacity of some microorganisms to accumulate heavy metals has been known since long. Heavy metals have become a high priority item in the environmental engineers' agenda because of the possibility of such bioaccumulation and subsequent biomagnification, in the ecological food chain. While engineering techniques, both chemical and physico-chemical, were applied for sequestering metals from environment, it was quite natural but innovative to think of microorganisms as potential and renewable adsorbents of heavy metals.

2.5.1 Early Observations of Metal Biosorption

The word biosorption may be new, but phenomenon is not. Wutrich (1892), working on the oligo-dynamic properties of copper and mercury on spores of a number of species of fungi, found that presence of copper could be readily demonstrated in spores which had been suspended in dilute solutions of copper sulfate. The author found

Table 2.6 Comparison of Treatment Technologies to Remove/Recover Metals*

Technology	Ability of the system to respond to variations in					
	Flow	Metal Concn.	pH	Suspended Solids	Other Metals	Expected Effluent
Precipitation						
Hydroxide	ok	no	no	ok	ok	2-5 mg/L
Sulfide	ok	no	no	ok	ok	2-5 mg/L
Ion-Exchange	no	ok	some	no	some	<1 mg/L
Membrane Processes	no	ok	some	no	some	5-25 mg/L
Adsorption	no	no	no	ok	ok	<1 mg/L

ok - System has the ability to respond to the variations
no - System has no ability to respond to the variations

* Brierly and others, 1986.

that the spores could remove so much copper from the applied solution that the presence of copper could not be demonstrated in the liquid phase.

2.5.2 Microbe-Metal Interaction

The interaction between microbes and metals has been and is being studied by scientists from areas as divergent as agricultural sciences to bio-inorganic chemistry. While the agricultural scientists were primarily interested in the microbicidal action of metals, Bioinorganic Chemists basically looked at the metal coordination geometry and environment in the metallo-proteins and their biological functions. While scientists from life sciences were concerned about the toxicological effects of heavy metals, its bioaccumulation and biomagnification, environmental engineers utilized this property of bioaccumulation for monitoring the heavy metal pollution in remote environs. Though different disciplines have variety of interests in metals, it is but natural that any environmental scientist interested in understanding the mechanism of biosorption should look at the common leads from all these areas.

2.5.3 Biological Monitoring of Metal Pollution

The phenomenon of biosorption was first put to creative use by scientists for monitoring traces of heavy metals in the environment. Algae, fungi and mosses are known to accumulate metals from air, soil and water. It is possible to determine the trace concentrations of metals in these environmental components by analyzing selected species of the above mentioned microbes which grow in the polluted area.

Godman and Roberts (1971) used mosses as the indicators of aerial metal levels, pioneering a novel technique of suspended moss (*Hypnum cupressiforme*) bags. Mosses have ion exchange properties similar to ion exchange resins and are thus suitable for collection and retention of metals dispersed in a fluid medium. Standard moss bags have been developed, which can provide rapid, reliable and inexpensive means of monitoring heavy metal pollution. Biomonitoring using brown algae *Ascophyllum nodosum* was reported by Huaga and others, 1972. Little and Martin (1974) reported the use of sphagnum moss suspended in fine nylon hair net for monitoring Zn, Pb, and Cd. The species took up to 155 μg/g/day, 59.3 μg/g/day, and 2563 μg/g/day of Zn, Pb and Cd respectively, averaged over a period of one month. Similar studies have also been reported by many other researchers (Ellison and others, 1976; Onianwa and others, 1976)

2.5.4 Metal Uptake in Activated Sludge Process

Many studies on metal balance in the wastewater treatment plants indicated that significant amounts of various metals are removed. (Brown and others, 1973; Lester and others, 1979; and Stoveland and others, 1979). A significant portion of this removal was accomplished in the activated sludge process. The metal removal was proposed to be a result of adsorption of dissolved metal or fine particulates on to the sludge flocs (Oliver and Cosegrove, 1974; Brown and others, 1973). The relationship between metal concentration in sewage and metal uptake by sludge flocs was investigated (Brown and others, 1973) and it was observed that for Cr, Cu, Zn, Pb and Cd, within non-toxic range (ie., < 2 mg/L), the metal uptake was directly proportional to the concentration. The kinetic study indicated a

rapid uptake of metals in the first 3 to 5 minutes followed by a very slow uptake in the next three hours and near total equilibrium in about two weeks. (Neufeld and Herman, 1975). Yet another significant observation was that biomass made non-viable by sterilization at 121 ^{o}C or blending decreased the metal uptake capacity (Cheng and others, 1975). They attributed this to the alteration occurring on the cell surface due to the harsh treatments imparted.

The aqueous chemistry of metals also exert significant impact on its uptake. It was suggested that of the total metal present, dissolved metal was removed to a lesser extent compared to other forms, resulting in a net increase in the proportion of dissolved metals to total metals in the effluent (Oliver and Cosegrove, 1974). As a result, metals having a low proportion of dissolved metals in the pH range 7-9 (normal pH of domestic wastewater) were more efficiently removed. In the case of chromium, the oxidation state has been found to affect the removal. While 70 to 90 % of trivalent chromium is removed, the typical values for hexavalent chromium were only around 20% (Stoveland, 1970). The presence of strong chelating agents like EDTA, fulvic and humic acid greatly decrease the metal uptake as the metals apparently have higher affinity for these chelating agents than for the sludge biomass (Cheng and others, 1975).

2.5.5 Metal Uptake by Algae

The uptake of cadmium and zinc by *Chlorella homosphare* cells were tested under laboratory conditions over a concentration range from 0.5 to 14.0 mg/L by Costa and Leite (1990). While there were two distinct phases of adsorption for cadmium, the zinc uptake was a continuum which the authors attributed to the metabolic

functions of zinc. At higher concentration of cadmium the growth of algae itself was affected.

Selective recovery of gold and other metal ions by algae *Chlorella vulgaris* was studied by Darnall and Others (1986). Heavy metals like Cr, Ag, Co, Ni, Cu, Zn, Au, Hg and U were adsorbed in a pH range of 5-7, the uptake capacity reaching up to levels of 10% of the dry weight of the algae. Most metals except Au, Ag and Hg could be recovered by lowering the pH of the reaction mixture, which could facilitate an elution scheme for selective recovery of these metals. Gold, silver and mercury could be recovered by exposing the algae to sulfur containing ligands like thiourea or mercaptoethanol (Darnall and others, 1986). An interesting observation by the authors was that algae made non-viable by boiling in water exhibited equally impressive metal uptake, which they ascribed to passive adsorption onto the cell walls.

The interaction of Cu(II) and Cd (II) with algal surfaces was evaluated by an innovative titration method by Xue and others (1988). Using a voltametric method capable of analyzing the metal in the presence of algal cells (*Chlamydomonas rheinhardii*) they found that the algal cells can uptake metal in competition with the natural complexing agents present. Most of the algae were alive after the metal uptake, their motility however was reduced.

2.5.6 Metal Uptake by Bacteria

Beveridge and Murray (1977) conducted the classic experimental work on aqueous metal complexation by bacteria. They showed that isolated cell walls of *Bacillus subtilis* sequestrated substantial amounts of Mg^{2+}, Fe^{3+}, Cu^{2+}, Na^{+} and K^{+} from dilute

aqueous solutions. Numerous studies have since identified a number of potential bacterial species capable of accumulating metals from aqueous environment. A strain of *Pseudomonas putida*, resistant to low environmental concentration (0.25 mM) of cadmium was adapted in the laboratory to higher levels (3 mM) in a chemically defined medium (Higham and others, 1985). The uptake of heavy metals copper, cadmium cobalt and nickel by extracted *Klebsiella aerogens* was reported by Rudd and others (1984). The authors also estimated the conditional stability constants for the complexes formed and found the maximum affinity for copper and least for cadmium.

2.5.7 Metal Uptake by Fungi

Heavy metal uptake of several species of fungi were studied by Gadd and Lousie (1988). The species employed were *Aureobasidium pullalans* and *Cladosporium resinae*. There was a distinct two phase uptake of heavy metals, the first consisting of metabolism independent cell wall uptake followed by a energy dependent cellular uptake. The latter was affected by low temperature, absence of energy source and metabolic inhibitors. Removal of cadmium by nine different species of fungi was investigated by Huang and others (1988) in both batch and continuous reactors. Biomass grown in the laboratory was harvested and stored in freeze dried condition. The uptake was found to be one controlled by adsorption and not by surface precipitation. Both fresh and freeze dried biomass gave comparable metal uptake. Under identical conditions the biomass also took up heavy metals Cu, Pb, Zn and Co.

2.5.8 Metal Uptake by Non-Viable Biomass

A significant observation derived from biosorption studies employing live microbial cells was that most of the microbes retained much of their metal uptake capacity even when deactivated by physical or chemical treatment. Though the live microbes may have energy mediated metal uptake mechanism over and above those possessed by deactivated microbes, the overriding difficulty of maintaining conditions conducive for maintenance of life of biomass within the treatment unit, prompted increased effort into the utilisation of nonviable organisms for metal uptake.

The mycelial mass produced as byproduct of citric acid industry (*Aspergillus niger*) was utilised for uptake of metal ions by Muzzareli and others (1980). The mycelial mass obtained after a mild alkali wash to make it non-viable was further imparted severe chemical treatments with concentrated alkali at elevated temperatures and a series of adsorbents were produced. These materials, when contacted with various heavy metals could take up most of the metals to levels exceeding 80% of influent at an adsorbent concentration of 4 g/L and an adsorbate concentration of 0.5 mM. The study identified an important potential of possible usage of byproduct biomass from fermentation units as biosorbents for pollution control and their possible improvement by chemical pretreatment.

Tsezos and Volesky (1981) reported the comparative evaluation of inactivated biomass originating from different fermentation processes and biological treatment plants for their capacity to take up radioactive nuclides uranium and thorium. All biomass were contacted with adsorbates under identical conditions. Biosorption isotherms

were compared with the performance of anion exchange (IRA 400) and activated carbon (Filtrasorb 400). The study identified *Rhizopus arrhizus*, a fungal culture which has since proven itself as an excellent biosorbent (Tobin and others, 1987), for a number of heavy metal ions. This study convincingly proved the superiority of biosorbents over ion exchange resins and activated carbons, both of which have been employed for treatment of metal bearing effluents. No chemical pretreatment of biomass was attempted to verify wthether such treatments could enhance the uptake potential of *R. arrhizus*. The study also did not report the details of the physical characteristics of the biomass which are important for continuous flow application. Later reports, however, indicate that *R. arrhizus* need to be either immobilised onto some solid matrix or the necessity of deploying specialised reactor configurations in place of simple reactors (Volesky, 1987).

A commercial biosorbent for metal bearing effluent treatment was developed and marketed by Advanced Mineral Technologies Inc., (Brierly and others 1986). This metal recovery agent was prepared and activated by a proprietary process. The agent was thereafter dried and ground to granules of required size. This material was capable of recovering substantial amounts of metal cations (Ag 86 mg/g, Cd 214 mg/g, Cu 152 mg/g) and could recover gold from jewelry effluents even in the presence of cyanide. The granules could be digested in nitric acid and precious metals could be recovered.

The metal uptake of a number of fungal biomass deactivated by chemical pre-treatments and high temperature was evaluated by Townsley and others (1986).

The metal uptake potential by green, brown and red marine algal species which were sun dried and powdered prior to adsorption experiments was evaluated and compared with that of other reported biosorbents by Kuyucak and Volesky (1989a). They found that algal biomass of *Ascophyllum nodosum* and *Sargassum natans* out performed ion-exchange resins in sequestering cobalt and gold from solutions.

2.5.9 Immobilized Cells for Biosorption

As obvious from the above, many potential biosorbents are present in the microbial world and one needs to identify them. However, to be practical on a field scale, it should be possible to develop some effective solid-liquid contact / separator system. Immobilization of microbes onto solid matrices is such a method suggested to circumvent the problems of solid-liquid contact/separation.

Polyacrylamide gel immobilized cells of *Citrobactor* sp. scavenge divalent heavy metals with high efficiency from simulated wastewater flows (Macaskie and others, 1987). The optimal environment for the metal scavenging has been identified as a combination of pre-growth condition of the cell and composition of the challenge solution. Of critical importance is the presence of an organic phosphate in the flow. High removal was observed for cadmium from flows supplemented with glycerol 2-phosphate (Macaskie and others, 1987). The uptake potential was related logarithmically to flow rate decreasing with increase in flow rate within a range of 30-100 mL/h. The columns were able to withstand up to a pH of 5 for two days with only 15% drop in metal uptake. Cadmium accumulation was also affected by increasing levels of chloride and cyanide ions with similar degree

of inhibition by 5 mM of CN^- as observed using 250 mM of chloride ions. Extremely high levels of uranium uptake (9000 mg of Uranium/g of sorbent) was observed when an immobilized column was challenged with flow containing 0.84 mM uranium, buffered at pH 4 for ten days (Macaskie and Dean, 1985). The adsorbed uranium could be desorbed by employing citrate buffer (pH 4.0).

Immobilisation of *Pseudomonas aeruginosa* on different ion exchange resins was evaluated by Ligy (1993). Both anion and cation exchange resins were used and quantitative enhancement of metal uptake upon immobilisation was demonstrated.

An immobilization medium studied extensively in biosorption studies is the alginate matrix (Costa and Leite, 1991; Wilkinson and others, 1989; Jang and others 1991). The process typically involves growing the cells in a controlled culture medium in the laboratory and harvesting them by centrifugation. These harvested cells are mixed with sodium alginate and sodium or calcium chloride, agitated extensively and extruded through a syringe into a solution containing sodium/calcium chloride salts. The beads formed were washed, packed in a column which was thereafter challenged with the target metal and adsorption achieved.

Costa and Leite (1991) studied the uptake of zinc and cadmium by *Chlorella homosphaera* immobilized on calcium alginate matrix. The column could achieve almost 100% removal of these metals from an initial concentration range of 20 to 41 mg/L for cadmium and 75 to 720 mg/L for zinc. When a mixture of metals were employed as influent solution, a decrease in the rate of adsorption was observed with lower metal concentrations. It was also observed that zinc was not so

efficiently extracted from the solution in the presence of cadmium indicating preferential uptake of metals.

Cellular accumulation, transformation and subsequent volatalisation of metallic mercury by alginate entrapped *Chlorella emersonii* was reported by Wilkinson and others (1989). Immobilized cells accumulated significantly more mercury than free cells. Substantial volatalisation of mercury was observed in the initial phases of the uptake but a part of the volatalised mercury reentered the aqueous phase when the experiment was conducted in a closed bioreactor.

High uptake of metals by the alginate matrix itself was observed when a viscous solution of alginate was directly dispersed into copper solution or when calcium alginate beads were agitated with copper solution (Jang and others, 1991). The copper adsorption onto alginate was to the tune of 100 mg/g when alginate was directly dispersed into a copper solution (60-200 ppm). In the case of algae (*Microcystis)* immobilized beads, the binding capacity was to 360 mg/g as predicted from isotherm studies. The conditional stability constant of alginate as well as algae-alginate beads showed a higher affinity for algae immobilized beads.

The immobilisation of twenty five isolates of soil bacteria onto many types of solid supports (rashing rings, cinder, silica gel, and polyacrylamide) and their subsequent utilisation for copper uptake was reported by Saxena (1993). The most dramatic increase of metal uptake was observed when the cells were immobilised on silica gel while virgin silica gel has only negligible metal uptake capacity.

Apart from algae and bacteria, fungal biomass also has been employed in immobilized bioreactors for removal and recovery of heavy metals (Lewis and Kiff, 1988; Zhou and Kiff, 1991; Tsezos and Deutschmann, 1992). *Rhizopus arrhizus*, a fungal biomass immobilized on reticulated foam biomass support was used for batch and column experiments (Zhou and Kiff, 1991). The pH of the medium was shown to influence the metal uptake significantly with the optimum being in the range of 6-9. Column residence time did not affect the uptake efficiency over a broad range but below a critical value uptake dropped dramatically. This minimum time, the authors concluded, is the residence time required for the copper ions to reach and interact with the binding sites. The uptake of copper was affected by the presence of cadmium indicating certain level of specificity. Other cations affected the uptake to varying degrees depending upon the affinity of the fungal biomass to these metals, which was found to be function of electronegativity and ionic radii. The exhausted column could be regenerated by eluting with 0.1 M HCl and the metal concentrated over 11 times. A similar attempt has also been made by Tobin and others (1987). Anionic ligands interfered with the uptake in accordance with their affinity towards the metal vis-a-vis the affinity of the metal towards the biosorbent.

Microfungal mycelia from different species were made into wet laid papers by an innovative casting method by Wales and Sagar (1990). The mycelial paper had breaking strength comparable to that of Whatman No.1 filter papers. The mycelial papers were contacted with metal in a dynamic flow mode. The effluent was allowed to flow through three layers of mycelial filters and the concentration of metal in the

effluent was monitored. In another contacting method, the mycelial papers were soaked overnight in copper sulphate solution after which, the paper was separated and checked for bound copper. The papers were found to be an effective method of immobilization and the material exhibited good potential for different metal ions (Ag, Zn, Pb, Ni, Co, Cd, Fe, and Cr).

In the case of viable biomass, while immobilisation definitely improves the process adaptability, the microbial mass need to be replenished once the biomass has reached its saturation capacity. This is unlike the case of other pollutants where an immobilised system can go for many cycles. To make this process more attractive, an elution technique capable of bringing out the adsorbed metal without affecting the viability of the microbe need to be designed.

2.6 MECHANISM OF BIOSORPTION

An understanding of the mechanism of biosorption is essential from both theoretical and practical point of view. While the fundamental question, why do one microbes sequester metals while most others do not, ought to be answered, an understanding of the mechanism can greatly aid the design and maintenance of reactors utilizing the biosorption phenomena. It may also be possible to enhance the metal uptake by selective pretreatments to the sorbent or sorbate solution, or providing favorable environmental conditions.

It is but natural that a process so ubiquitous as microbial metal accumulation should have been studied extensively. However the explanations given for the uptake varies from simple physical adsorption to intra cellular precipitation aided by metabolic transport of metals across the cell membrane. While it is possible

that all such processes may be acting individually or in combinations, a well founded theory of such an uptake supported by experimentation is yet to evolve. Some of the metal uptake processes studied in detail are discussed here.

2.6.1 Metal-Protein Interactions

Since many metals are associated with different enzymes responsible for various life functions, the co-ordination geometry and environment of such metals in the biological systems have been studied extensively (Spiro, 1976) This reported metal-protein interactions have been proposed as the major mechanism behind biosorption by different workers (Esser and Brunnert, 1986; Brierly and others, 1986; Friis and Keith, 1986). However it was observed that the amount of these metals required for metabolic functions are at least two orders of magnitude less than that of the reported uptake which clearly implies that the metal uptake cannot be by an intra cellular protein with a biological function. It is however possible that the metal is bound to cell wall proteins.

2.6.2 Intracellular and Extracellular Polymers

Several possible mechanisms of metal accumulations in activated sludge have been proposed, they are;

1. physical trapping of precipitated metal in sludge-floc matrix
2. binding of soluble metal in extra cellular polymers
3. accumulation of soluble metals by the cell

Cheng and others (1975) proposed that at high metal concentrations, when large proportions of metals are present as precipitates, these may be physically entangled in the biological floc matrix. A number of sites for metal retention in activated sludge

were also suggested by mechanisms like ion-exchange, sorption, chelation or precipitation (Brown and Lester, 1979).

Binding of soluble metals to extra cellular polymers of activated sludge has been studied in detail. Many species of microbes isolated from activated sludge have been shown to produce extra cellular polymers (Sutherland and Wilkinson, 1971). These polymers may be in the form of loose slime or capsules or micro-capsules which adhere to the cell wall. In ASP, slime polymers remain with the dissolved and colloidal part of an effluent, where as the capsular polymers remain attached to the flocs and settles (Brown and Lester, 1980, Fletcher and Beckett, 1987b). The extra cellular polymers are mostly made out of polysaccharides and may also contain uronic acid.

2.6.3 Surface Binding

Tsezos and Volesky (1982 a and b) reported the results of extensive research work carried out to elucidate the mechanism of biosorption of uranium and thorium by a fungal biomass *R. arrhizus*, which was shown to have excellent biosorption properties. Based upon sophisticated instrumentation like EPR, IR, EDAX and electron microscopy, they had proposed a three stage process for metal accumulation. Firstly the metal coordinated with the chitin matrix which act as nucleation sites for further adsorption. In the third step the co-ordinated metal is hydrolyzed and deposited on to the cell wall leading to further metal uptake. The cell wall has been reported to be the site of metal uptake for common sea weed *Ascophyllum nodosum* adsorbing cobalt (Kuyucak and Volesky, 1989c). This study made use of TEM as well as EDAX studies to locate the site of interaction.

Metal-Algae interaction in a unialgal culture, *Stichococcus bacillaris* was investigated using ^{113}Cd nuclear magnetic resonance (NMR) studies (Majidi and others, 1990). By studying the competitive nature of adsorption for various heavy metals and the information about chemical shift from the nmr studies, the authors concluded that the dominant metal binding sites on the cell wall are most probably carboxilic groups.

Heavy metal binding to digested sludge and digested sludges treated with EDTA was reported (Alibhai and others, 1985). The complexation of lead, iron and chromium was not a function of temperature. The metal could not be desorbed by EDTA again suggesting stronger interaction.

2.7 FIELD APPLICATIONS OF BIOSORPTION

Though one decade is not a substantially long period for a process to evolve from lab to field, the field applications of biosorption has kept pace with its laboratory developments. Currently there are at least three enterprises which markets biosorbents (Volesky, 1990)

1. B.V.SORBEX, Inc., in Montreal, Canada, is currently developing a series of biosorbents for a spectrum of metals and range of concentrations.
2. Bio-Recovery Systems, Inc., in Las Cruce, USA, developed a biosorbent by immobilising a biomass of fresh water alga *Chlorella* in silica or polyacrylamide gels.
3. Advanced Mineral Technologies Inc., USA developed a broad based metal sequestering biosorbent based on *Bacillus* sp.

Biosorption being similar to ion-exchange, the processes developed are also similar in nature. A typical metal uptake cycle involves the following steps:

1. Contacting the metal bearing effluent and biosorbent in an appropriate reactor configuration
2. Separating metal laden biosorbent from metal free effluent
3. Recovering the metal from biosorbent by eluting with an appropriate desorbing agent. In a few cases the biosorbent is digested to concentrate the metal to achieve economically recoverable levels
4. Regenerating the biosorbent and reuse the biosorbent.

In accordance with the process mechanism, kinetics, uptake capacity and the physical characteristics of the biosorbents, many reactor configurations have been suggested in both batch and continuous flow mode. The typical reactor configurations suggested are

1. Batch stirred tank reactor
2. Continuous flow stirred tank contactor
3. Fixed packed bed contactor
4. Pulsating bed contactor
5. Fluidised bed contactor
6. Multiple bed contact arrangement

Though there have been number of reports regarding metal uptake potential of both live and dead microbes, it can be noticed that all field scale units thus far have come up employing the dead microbes. This preferential development of dead microbes is a natural consequence of the distinct advantages they provide by them in the

treatment of a toxic pollutants. The advantages employing dead microbes are

1. They do not need an effluent supplemented with nutrients and many nutrients interfere with metal uptake. Thus dead microbes achieve a reduction in cost and increase in efficiency.
2. Process need not be maintained at optimum physiological conditions (pH, temperature, oxygen concentration etc.) and hence these parameters can be optimised for maximum metal uptake
3. The metal adsorbed onto the biosorbent can be recovered by any appropriate eluting agent irrespective of the potential toxic action of the eluting medium.
4. Physico-chemical treatments as may be needed to maximise metal uptake can be given to the biosorbent
5. Since the adsorption action is not guided by any metabolically mediated process, better mathematical description of the process is possible.

2.8 RARE EARTHS AS RESOURCES AND POLLUTANTS

2.8.1 Definition

Rare earths are elements in the Periodic Table occurring between elements 58 and 71 both inclusive. They are La, Ce, Pr, Nr, Pm, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb and Lu. For these elements as the charge of the nucleus increases, the balancing electron will fill in the inner incomplete 4f sub shell. Since 4f electrons are well shielded by the 5s5p subshells, they play almost no role in the valancy forces. All rare earths have three electron in their valancy shells and since these electrons are responsible for most physical and chemical properties, the rare earths closely resemble each other (Jogerson, 1987)

Rare rare earths are neither rare nor they are earths (Gschneidner, 1981). Of the 83 naturally occurring elements rare earth elements fall into the fiftieth percentile of elemental abundance. Cerium, the most abundant, ranks 28th and Terbium, the least abundant, ranks 63rd. Rare earths are 10 to 15 times more abundant than thorium in monazite which is an ore of both rare earths and thorium.

All rare earths are metals and the misnomer earths came out of a historical accident. The first observation concerning their existence was made by Arrhenius in 1794 (Szymanski, 1987) when he reported the discovery of the earth yttria, present in certain Swedish ores. During eighteenth century, a number of oxides were isolated and were believed to be elements (Gschneidner, 1984). They had alkaline properties, and the scientists at that time were unable to melt them or observe any changes occurring in them when heated. Since yttria resembled the common earths such as lime and alumina, it was referred to as a rare earth.

2.8.2 Aplications of Rare Earths

Because of their unique electronic structure, rare earth elements have specialised applications in the area of optical, electronic and magnetic instrumentation. Many of the rare earth ions are used as activators (in phosphors) and lasing ions (in lasers). Europium (III) is the activator for primary red color in color televisions (Tecotzky, 1987). Other luminescence applications of rare earth elements are listed in Figure 2.4. In the electronics and magnetic area, the most important rare earths compounds, from an application view point, are garnet based material, yttrium iron garnet

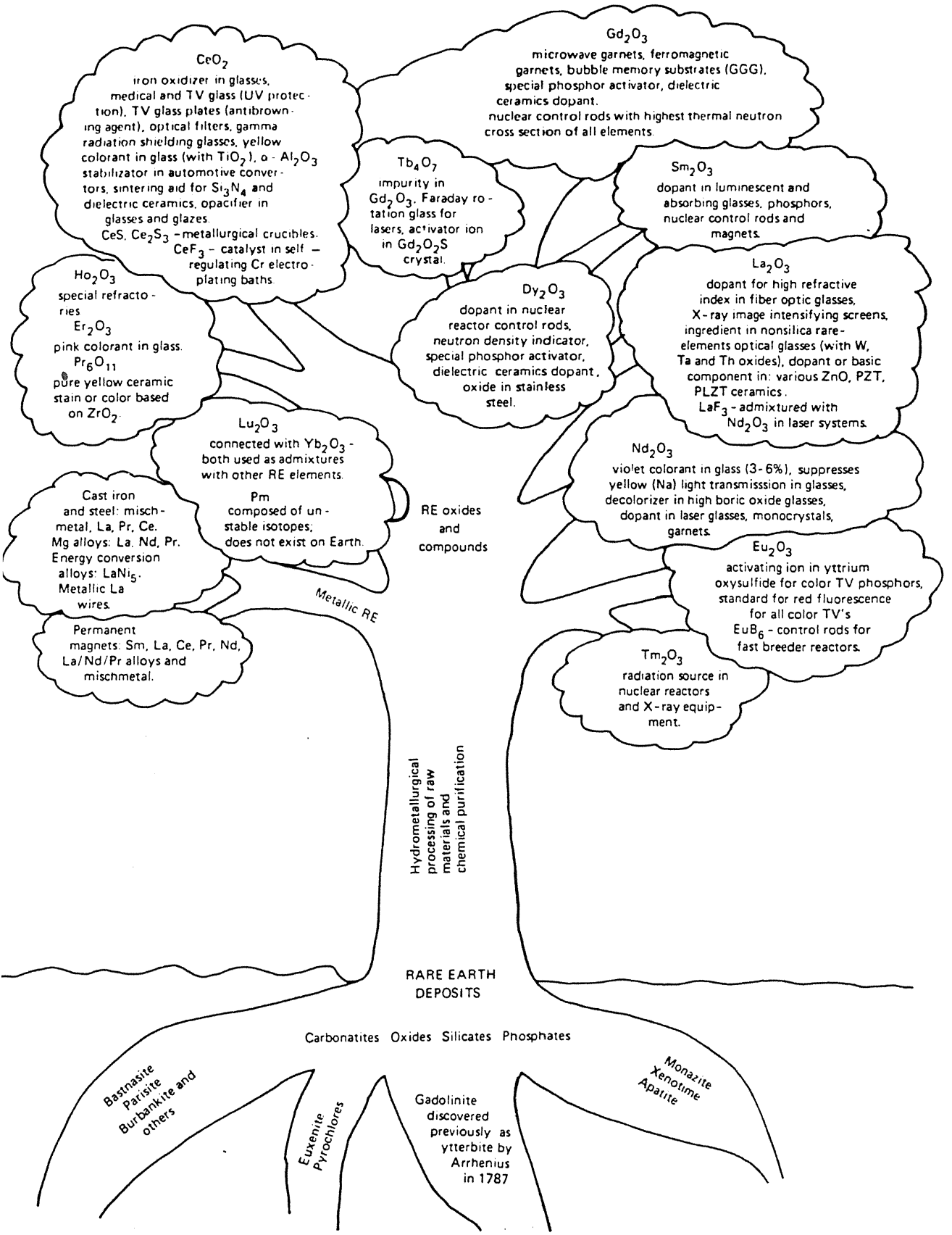


Fig. 2.4. Applications of Rare Earth Elements. (Ref.: Szymanski, 1987)

(YIG) and gadolinium gallium garnet (GGG). The YIG materials are used in the polycrystalline form in a variety of microwave devices including attenuaters, isolators, phase shifters, power limiters and switches.

2.8.3 Rare Earth Ore processing

The three major minerals of rare earths are bastnastite, monazite and xenotime, of which the first two accounts for 95% of the rare earths being produced (Gschneidner, 1981). Bastnastites are fluorocarbonates whereas monazite and xenotime are rare earth phosphates. Monazite account for about 40% of the world utilisation. India has a significant deposits of rare earth in the form of beach sand. In the present study, monazite processing industry is therefore identified as an ideal site to verify the practical potential of biosorption process and hence a detailed description of the processing of monazite is warranted.

Monazite is a rare earth phosphate containing 5 to 10 % of ThO_2. Since thorium is a fissionable material it is removed and stockpiled before exporting rare earths. Because of the natural radioactivity of thorium, there are stringent environmental regulations for its processing.

The beach sand which contain monazite (generally 1-20 %) is recovered by dredges and is separated from other impurities by a combination of gravity, electromagnetic and electrostatic techniques, and is cracked by either an acid process or a basic process. In the basic process, which is used more extensively, the finely ground monazite is mixed with 70 % NaOH solution and heated in an autoclave at 140-150^{o}C for several hours. After the addition of water, the

soluble Na_3PO_4 is recovered as a byproduct from the insoluble $R(OH)_3$, which still contains 5-10 % Th. The pH is brought down to 3-4 and the soluble RCl_3 is separated from the insoluble $Th(OH)_4$ by filtration. The thorium free rare earth is converted into usable form (hydrated chloride, anhydrous chloride, fluoride, carbonate, hydroxide, or oxides) or is further processed to obtain individual elements (Gschneidner, 1981).

Separation and purification of individual rare earths are done either by a solvent extraction method or by an ion exchange method. Ion exchange can purify all of the rare earth elements to 5 nine purity (99.999 %) where as the solvent extraction method is yet to achieve this level of purity. The ion exchange technique for rare earth separation is elaborated here.

In the ion exchange process, a metal ion, R^{3+} in solution exchanges with a proton on a solid ion exchanger, a synthetic resin or a natural zeolite. The tenacity with which the cation is held by the resin depends upon the size of the ion and its charge. However no separation of the rare earth is possible because the resin is not selective enough. By introducing a complexing agent, A^*, separation is possible if the equilibrium constant for the reaction

$$R + 3A^* \rightleftharpoons RA_3^* \qquad 2.3$$

varies sufficiently from one rare earth to another for separation to take place. Furthermore in the RA^* system there should only be one complexing species.

In the ion exchange process the resin bed is prepared by passing an acid through the column, then the resin bed is loaded with a mixed rare earth solution which contains the complexing agent and a

retaining ion such as Cu^{2+} or Zn^{2+}. The retaining ion is needed to prevent the first rare earth ion from spreading out and being lost during the separation process. An eluent, generally NH_4^+ is needed to push the rare earths through the ion exchange columns. The most stable compounds comes out first, ie., the copper or zinc complex followed by Lu, Yb, etc., and finally La. The rare earths thus obtained is precipitated by using an oxalic acid solution and then is converted into required chemical form.

The process effluent from rare earth processing contains heavy metals (Zn & Pb), phosphates, and fluorides in addition to traces of the processed rare earth elements. In the Indian scenario particularly, since monazite contains thorium in addition to the rare earth elements, the process effluent will also contain traces of this radio-active nuclide. Because the concentration of the target elements are very low, conventional processes like chemical precipitation etc., can not be economically/efficiently employed for the effluent treatment.

2.8.4 Toxicological Information

Rare earths belong to the category of heavy metals whether taken from the electronic configuration point of view or the toxicity angle. However, not much information is available on the adverse environmental impacts of rare earths. This reflects the confined production and application of rare earths than their lacking any detrimental effects. Rare earths can degrade DNA molecule (Eichborn, 1965) and have reported to produce tumors at the site of interaction (Ball and others, 1970). Rare earths also bond with plasma proteins and accumulate in bones (Ando and Hisada, 1972).

Lanthanum levels are consistently high in the blood of cancer patients, though a direct convincing cause-effect relationship is yet to evolve (Flessel and others, 1980).

2.8.5 Biosorption in Rare Earth Processing ?

Rare earth industry appears to be an ideal candidate for trying out biosorption process. Firstly, the effluent consists of a mixture of elements, each of which is a pollutant and hence need to be removed. Biosorbent being versatile, a single adsorption bed can be employed for all the metals. Secondly, the effluent concentration of the elements is relatively small that the traditional methods may not be cost effective. Thirdly, the input to the industry being concentrated metallic solution, the elements if concentrated can be directly pumped back into the process stream and no separate recovery unit is necessary. Most importantly, rare earth industry being familiar with ion exchange process, the technology shock while introducing this new method will be minimal unlike in the case of metal processing or plating industries.

2.9 SUMMARY

Heavy metal pollution continues to be one of the major challenges for environmental engineers because of their ubiquity and multiplicity of impacts. Metals are essential ingredients of modern way of living and the possibility of their complete elimination is remote. Further, it is clear that some of the metals are essential for biological systems.

A number of treatment technologies are being employed for management of heavy metals present in industrial effluents. Destructive methods like precipitation, the traditional favorites are

becoming obsolete to meet the more stringent standards. Recovery methods like electrodialysis, ion exchange etc., though effective at high metal concentrations are economically infeasible at lower concentrations. Search for new technologies are thus continuing for both economical and technical reasons.

Of the newer technologies for heavy metal management, biosorption undoubtedly has emerged as the most promising. This technology, in which microorganism like bacteria, algae or fungi are being employed for the uptake of heavy metals from low level industrial effluents have been developed from laboratory to field scale in a short time span of about a decade. In most cases the microorganisms are immobilised onto some solid matrix to attain effective solid-liquid separation. Non-viable species and cellular component isolates also have been employed as biosorbents and found to be effective. The investigations pertained to laboratory grown or byproduct of industrial fermentation process. The vast biodiversity which is recognised as treasure of modern biotechnology is yet to be exploited. Investigations are required to use the widely and wildly growing macrofungi for the metal removal.

3. SCOPE OF THE INVESTIGATION

A comprehensive survey of literature presented in the previous chapter clearly brings out the fact that there is an urgent need to develop newer and efficient techniques for the control of pollution of aqueous environment by metals. It has also established the fact that biosorption has emerged in the last decade as a promising alternative to the existing metal bearing effluent treatment technologies.

The biological diversity available in tropical forests have been identified as an extremely fertile field for further developments in biotechnology. It has been identified in many areas of biotechnology that wild species have many special and useful characteristics not generally possessed by lab grown species (Anonymous, 1986). It is therefore imperative that the possible presence of potential biosorbents for metal binding in wild species be explored.

In addition to the identification of potential biosorbents, the possible improvement of these biosorbents by chemical treatments need to be studied. The elucidation of mechanism of biosorption by these species is also of utmost importance from both fundamental and application point of view. The specificity as well as versatility of the biosorbent need to be evaluated before conducting scale up studies.

The present investigation was conducted along the following lines

1. Screening of nine species of saphrophytic macrofungi, which grow widely and wildly in tropical regions for their metal uptake potential. This was done by batch adsorption experiments.
2. Chemical alteration of the selected biosorbents for enhancing their uptake as well as the properties for use in continuous flow systems.

3. Evaluation and comparison of various physico-chemical and engineering properties of the biosorbents prepared from the native biomass.

4. Elucidation of metal uptake mechanism of biosorbents using a combination of biochemical and spectroscopic techniques. The biochemical techniques are aimed at a sequential elution of different cellular components to isolate and possibly identify the coordinating component. This is supplemented by employing spectroscopic methods such as (a) Energy Dispersive Analysis by X- rays (EDAX) (b) Electron Paramagnetic Resonance (EPR), (c) Transmission Electron Microscope (TEM) and (d) Infrared (IR).

6. Evaluation of the response of the biosorbent to a wide variety of aqueous chemical environments.

7. Kinetics of the adsorption is of utmost importance from a practical point of view. Kinetics studies conducted include (a) kinetics of adsorption and desorption (b) Evaluation of rate limiting step from kinetic data.

8. The behavior of an adsorbent in a packed bed need to be evaluated from an engineering point of view for deciding reactor configuration.

9. To test the developed biosorbent, rare earth processing industry was selected as a model. Bench scale models of packed bed reactors were operated using rare earths as adsorbates to obtain details regarding the following (a) Adsorption-desorption behavior of the biosorbent (b) Separation between individual rare earths (c) Treatment of simulated effluents from monazite processing industries.

4. MATERIALS AND METHODS

4.1 MATERIALS

4.1.1. Glassware

All glassware used in the present study were manufactured by M/S Borosil Glass Works Ltd. (Bombay, India) and marketed under the brand name 'Borosil'. All glassware were washed with liquid soap followed by washing with tap water and distilled water. The glassware for adsorption study were finally immersed in the adsorbate metallic solution (1 mM) for 24 hours and washed in distilled water.

4.1.2 Water

All chemical solutions were prepared in water freshly distilled in the laboratory. The average pH of the distilled water was around 7.0.

4.1.3 Chemicals

All chemicals used were of analytical reagent grade except in specialized experiments (Electron microscopy and Infrared) where special grade chemicals were used. Rare earths used for the present study was of triple nine (99.9%) purity grade as obtained from Indian Rare Earths Ltd. (Udyogamandal, India).

4.1.4 Fungal Fruiting Bodies (Mushrooms) for Biosorption

Wood-rotting fungi grow prolifically in humid temperate climate and the maximum growth of fruiting bodies has been reported to be during the post monsoon period (Smith, 1963). The mushrooms were therefore collected during the post monsoon period (September-October) from forests in Kerala (India). The fruiting bodies were detached from the rotting wood, washed in water and was sun dried for two days before transporting to the laboratory.

4.1.4.1 Sample Preparation for Identification

Representative specimen from visibly different groups of mushrooms were soaked in 1% formaldehyde solution for 24 h to prevent biodegradation. These samples were then dried at 40^{o}C for 24 h, packed in polyethylene bags and were despatched to the Royal Botanical Garden (Kew, U.K) for identification. Once the genera and species level identification was obtained, further taxonomical information was collected from standard text book (Talbot, 1971). The detailed classification is presented in Table 4.1.

4.1.4.2 Adsorbent Preparation

Biosorbents were prepared from the mushrooms by pulverizing the fruiting bodies. The fraction of particles between 1200μm and 600μm (geometric mean size 848 μm) was collected for adsorption study as it facilitated easy handling.

A number of chemical pretreatments which have been reported to be effective for enhancement of metal uptake were employed for the selected biosorbents. The starting material for chemical pretreatments was the pulverized biosorbent of geometric mean size 848 μm. The raw sorbent which is subjected to various pretreatment is designated as M_1

4.1.4.3 Nitric Acid Formaldehyde Treatment

Nitric acid-formaldehyde treatment was given to biosorbent M_1 as per the procedure laid out by Freer and others (1989). Ten grams of the biosorbent was mixed with 150 mL of 3% nitric acid and 0.25 mL 35% formaldehyde, and was boiled in a water bath for 15 min. Subsequently the mixture was cooled to room temperature,

Table 4.1 Taxonomical Classification of Fungi Used for Biosorption*

DIVISION	CLASS	ORDER	FAMILY	GENUS	SPECIES
Mycota	Basidiomycetis	Polyporales	Polyporaceae	Coriolopsis	strumosa
Mycota	Basidiomycetis	Polyporales	Polyporaceae	Tramates	lactenia
Mycota	Basidiomycetis	Polyporales	Polyporaceae	Rigidoporus	lineatus
Mycota	Basidiomycetis	Polyporales	Polyporaceae	Daedalea	tenuis
Mycota	Basidiomycetis	Polyporales	Polyporaceae	Phellinus	xeranticus
Mycota	Basidiomycetis	Agaricales	Agaricaceae	Lentinus	strigosus
Mycota	Basidiomycetis	Aphyllophorales	Ganodermataceae	Ganoderma	lucidum
Mycota	Ascomycetes	Polyporales	Polyporaceae	Lenzites	malaccenis
Mycota	Ascomycetes	Polyporales	Polyporaceae	Rigidopoous	microporous

* Talbot, 1971

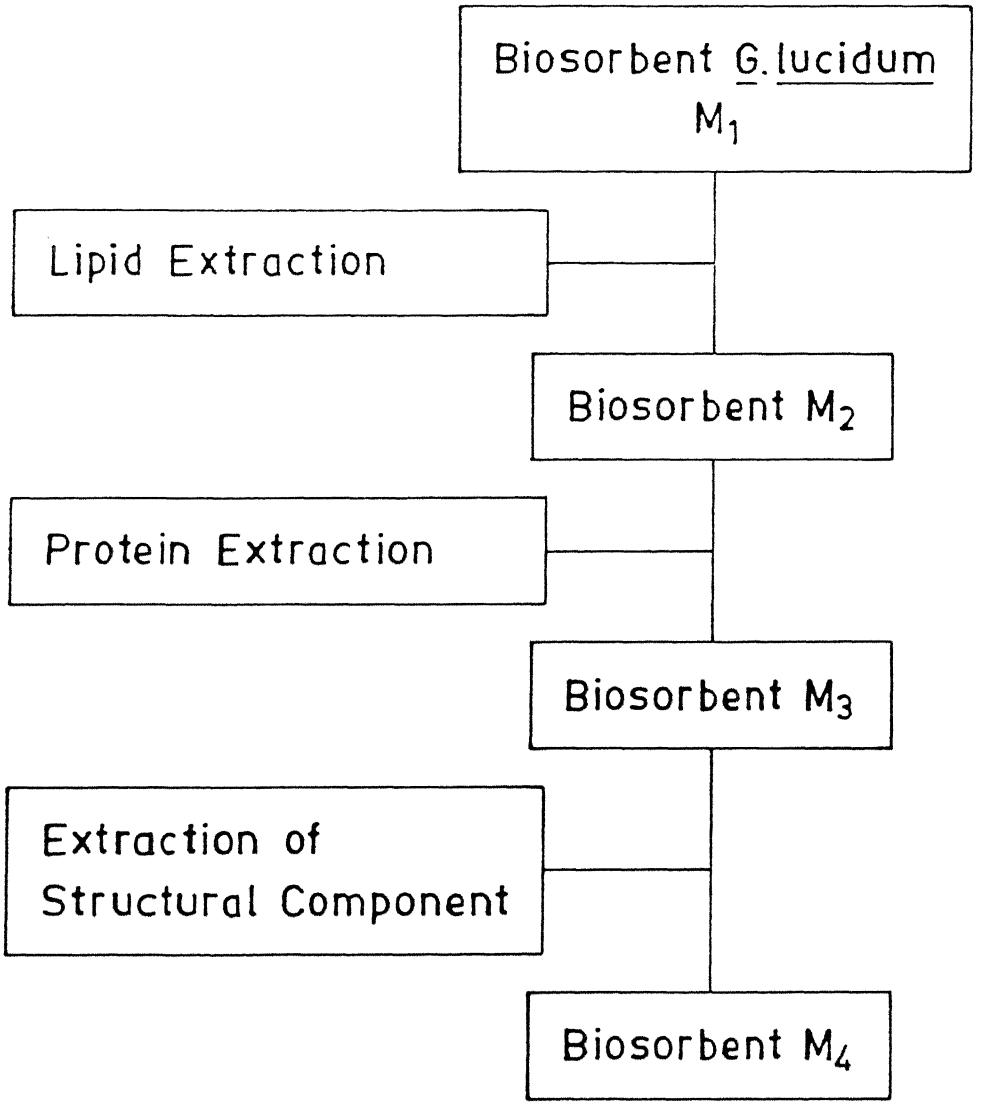


Fig. 4.1. Sequential Elution of Hyphal Cells of G.lucidum.

4.1.4.5.2 Protein Extraction

Extraction of protein was done by the method suggested by Smithes (1952). Twenty grams of the above adsorbent (M_2) was mixed with 100 mL of 2N NaOH at room temperature by constant stirring. The residue was separated, washed with distilled water till neutral pH was achieved. The residue was dried at 40°C for 24 h. The resultant residue is referred as M_3.

4.1.4.5.3 Hot Alkali Treatment

Muzzareli and others (1980) reported sixteen different ways of pretreating the mycelia of *Aspergillus niger* by hot concentrated alkali for the preparation of efficient biosorbents. A method which gave an optimum combination of yield and metal uptake was chosen in the present study. Twenty grams of the biosorbent, M_3, was mixed with 200 mL of 40% NaOH and the mixture was heated at 128°C for four hours. After this, the mixture was cooled to room temperature. This residue was separated by settling and washed thoroughly with distilled water till there was no change in pH. The mixture was dried at 40°C for 24 h.

4.2. METHODS

4.2.1 Characterization of Adsorbent

Since no standardized method was available to characterise the biosorbents, this work was undertaken as per the detailed specifications outlined in American Water Works Association manual (AWWA, 1943) for testing zeolites. These methods were appropriately modified whereever necessary and the details are presented in the following sections. Whenever there was no established methods, a testing procedure is designed to suit the requirements.

4.2.1.1 Water Soluble Matter

One gram of biosorbent was mixed with 25 mL of distilled water and the mixture was agitated employing a magnetic stirrer (Susmitex, Bombay, India). After 5 minutes, the mixture was filtered and transferred into a tared porcelain crucible. The filtrate was evaporated to dryness in a constant temperature oven at 105^{o}C for 24 h. The weight is reported as a percentage of the original weight.

4.2.1.2 Apparent Density (Dry)

The biosorbent was dried at 105^{o}C for 3 h and cooled to room temperature in stoppered bottles. Ten gram of this biosorbent was poured into a graduate (with glass stoppered top). The graduate was tapped till constant volume was attained and this volume was noted. Dry density of biosorbent is reported as g/L.

4.2.1.3 Apparent Density (Wet)

Ten grams of biosorbent was poured into a 100 mL graduate half filled with water. This was thoroughly mixed by inverting the stoppered graduate many times and later the sorbent was allowed to settle to a constant volume. The apparent density (wet) is reported as g/L.

4.2.1.4 Specific Gravity

Specific gravity of the biosorbent was calculated by measuring the volume of water displaced by 5g of the sorbent.

4.2.1.5 Hardness

The hardness of adsorbent was measured by agitating 5g of the sorbent (1200-600 μm) with 10g of glass beads

(2mm ϕ) in an end on end rotary shaker (Widson Scientific Works, Delhi) (30 rpm) for 30 min. The sorbent was thereafter sieved again through 600 μm sieve and the weight of particles retained in the sieve (>600 μm) was determined. The fraction of adsorbents which withstood this test is reported as a percentage.

4.2.1.6 pH of the Biosorbent

pH of an adsorbent was measured by determining the pH of distilled water with which the biosorbent was in contact under under specified conditions. To determine the pH, ten grams of the biosorbent was mixed with 100 mL distilled water and the mixture was boiled for 1 h making up the volume to 100 mL by intermittent addition of distilled water if necessary. The sorbent was separated by filtration and the pH of the supernatant was measured.

4.2.1.7 Resistance to Liquid Percolation

Resistance offered by the biosorbent to the liquid flow (Filterability) was determined in a down flow mode fixed bed reactor, for a constant static head of 750 mm. The column was 50 mm in diameter and was packed to a height of 600 mm with biosorbent. The maximum flow rate which could be maintained under these conditions is a measure of its resistance to flow and is reported as limiting flow. The head loss developed in the above column bed at a flow rate of 3.5 $m^3/m^2/sec$ was also measured and is reported as cm of head loss/cm of column length.

4.2.1.8 Thermal Stability

Experiments to determine the ability of the biosorbents to sustain high temperature and pressure were conducted.

Ten grams each of the biosorbent M_1 was heated in a controlled temperature hot air oven (PSI DT-VII Microtech, India) for 3 h. The adsorbents were thereafter cooled to room temperature, weighed and were employed in adsorption experiments.

Another 10 g each of the biosorbents was mixed with 50 mL distilled water and was autoclaved (Widson Scientific Works, India) at 1.089 kg/cm^2 (Gauge pressure) and 121^oC for 3 h. After this, the biosorbents were taken out by settling and dried at 40 ^oC for 24 h, weighed and were employed in adsorption experiments.

4.2.1.9 Leaching Characteristics

One major drawback of the biosorbents is the leaching of color causing organics into the aqueous phase. A quantitative evaluation of the leaching characteristics was undertaken by conducting a leachability test. One gram of biosorbent was agitated with 50 mL of distilled water on a rotary shaker for 24 hours. The biosorbent was separated by settling and the Chemical Oxygen Demand (COD) of the supernatant was determined as per Standard Methods (1989).

4.2.1.10 Ash Content

Ash content was determined as per Standard Methods (1989). One gram of the biosorbent was kept in a muffle furnace (SUNVIC, UK) at 550 ∓ 50^oC for 1 hour. The residue was cooled to room temperature in a desiccator and weighed. Ash content is reported as a percent of original weight.

4.2.2 Analysis of Metals

Analysis of the samples for various metals, other than rare earths and thorium, were conducted by employing Inductively

Coupled Plasma (ICP) atomic emission spectroscopy on a Labtam ICP AES (Labtam, Australia). The standards for analyses were prepared from electrolytic grade metal powder digested in nitric acid.

4.2.3 Analysis of Rare Earth Elements

Individual and mixed rare earth elements were analysed using colorimetric method developed by Onishi and Sekine (1972). To a 15 mL of sample or aliquot was added 50 % ammonium acetate solution or 4 M hydrochloric acid to bring the pH to 3.0 - 3.5. One mL of aqueous (0.1 % w/v) Arsenazo III was added and the volume made up to 25 mL. The absorbance was measured at 660 nm using a Systronics 106 (M/s Systronics, Ahmedabad) spectrophotometer. The calibration range was 0 - 20 mg/L. Qualitative analysis of rare earths praseodymium and neodymium was done by using a recording UV-Vis spectrophotometer (Shimadzu, Japan) as suggested by Stewart and Kato (1958).

4.2.4 Analysis of Thorium

Analysis of thorium was done by the spectrophotometric technique developed by Marcenko (1976) using Arsenazo III as the color developing agent. Arsenazo stock was prepared by dissolving 100 mg of arsenazo (III) in 20 mL 1 N NaOH, the pH was thereafter brought to 1.5 by concentrated H_2SO_4, the volume was thereafter made upto 100 ml by 0.1 N H_2SO_4. A working solution was prepared by diluting this reagent with 0.1 N H_2SO_4 (1 mL arsenazo (III) + 9 mL acid). To 1 mL of sample or aliquot was added 5 mL of arsenazo (III) reagent. The color developed after 15 min was measured at 656 nm. A calibration curve in the range of 0-20 mg/L was found most appropriate.

4.2.5 Adsorption Experiments

Adsorption experiments were conducted with a number of metals (from a spectrum of transition elements and lanthanides). While equilibrium study was conducted with all elements, studies to evaluate other adsorption behaviour (kinetics, effect of pH etc.) were done with a model element from each group. Copper (II) was used as a model transition element where as lanthanum and mixed rare earths (Composition given in Table 4.2) were employed as representative of rare earth elements. This was done because of availability of more literature data about these ions which facilitates better comparison.

Table 4.2 Composition of Mixed Rare Earths

Element	La	Ce	Nd	Pr	Sm	Eu Gd Tb Dy Ho Er Tm Yb Lu
Percent by weight	23	46	05	20	04	02

4.2.5.1 Kinetics of Adsorption

Reaction mixture for kinetics studies consisted of 100 mL adsorbate solution and 2.5 g/L biosorbent in all cases. The adsorbate concentration was 1.0 mM in the case of copper and lanthanum and 250 mg/L as rare earth chloride in the case of mixed rare earth elements. The pH of the solutions were maintained at 4.0 using 0.1 M acetate buffer. Adsorbents were added immediately before the sampling bottles were mounted on a rotary shaker (30 rpm). Reaction bottles were removed from the shaker at timings of 1, 3, 5,

10, 15, 30, 60, 120, 180 and 240 min, samples were immediately withdrawn and analyzed for residual metal concentration.

4.2.5.2 Effect of pH on Adsorption

Adsorption study was conducted over a pH range of 1 to 6, the pH adjustments were done by NaOH or HCl. No buffer was used as it was not possible to use a single buffer over the whole range. Further, different components used to make various buffers may themselves affect the adsorption of the metal to different degrees. The reaction mixture consisted of 100 mL of the adsorbate solutions (1 mM) adjusted to various pH and the adsorbent concentration kept constant at 2.5 g/L. The mixture was then agitated thoroughly on a rotary shaker for 3 h at 30 rpm following which the sorbents were separated and the solution was analyzed for Cu(II).

4.2.5.3 Equilibrium Studies

Adsorption equilibria were conducted with adsorbates Mn^{2+}, Cr^{3+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+}, Hg^{2+}, La^{3+}, Pr^{3+}, Nd^{3+}, Dy^{3+}, Eu^{3+}, Tb^{3+}, Y^{3+}, Sm^{3+}, Gd^{3+} and mixed rare earth chloride solutions. The initial concentration varied from 0.2 to 2 mM of the individual elements and 50 to 250 mg/L (as RCl_3) in the case of mixed rare earths. Reaction mixture consisted of 100 mL adsorbate solution buffered at pH 4.0 by acetate buffer (0.1 M strength, 2.5 mL in 50 mL adsorbate solution) and 2.5 g/L of adsorbent. The reaction time was maintained constant at 3 h and the mixture was agitated at 30 rpm on an end-on-end rotary shaker. Subsequently the adsorbent was separated by settling and supernatant analyzed for adsorbate concentrations.

4.2.5.4 Multi Metal Uptake Studies

To study the effect of competing ions, experiments were conducted with reaction mixture containing more than one adsorbate. The competing ion employed was lanthanum and the molar concentration of both elements (copper and lanthanum) was kept constant at 1 mM. After 3 h reaction time, the sorbent was separated by settling and samples analysed for Cu (II) and La (III).

4.2.5.5 Effect of Complexing Agents

The effect of a number of complexing ligands on the uptake of copper (II) by biosorbent was studied. The ligands studied, their concentrations employed and the salts used are presented in Table 4. 3. The sorbate concentration was 1 mM and sorbent concentration 2.5 g/L. The reaction bottles were mounted on a rotary shaker (30 rpm), agitated for 3 hours after which the sorbents were separated by settling and samples analyzed for copper(II).

Table 4.3 Complexing Agents Used in Metal Uptake Studies

Complexing Agent	Concentration mM	Compound
Acetate	1 - 10	$CH_3COONa.3H_2O$
Carbonate	1 - 10	Na_2CO_3
Citrate	1 - 10	$NaC_6H_5O_7\ 2H_2O$
EDTA	1 - 10	$[CH_2N\ (CH\ COOH)_2]_2$
Cyanide	1 - 10	NaCN
Nitrite	1 - 10	$NaNO_2$
Perchlorate	1 - 10	$NaClO_4$
Persulphate	1 - 10	$(NH_4)_2\ S_2O_4$
Tartrate	1 - 10	$Na_3(PO)_4$

4.2.5.6 Batch Desorption Studies

Batch desorption studies were conducted with biosorbent M_1 after adsorption of copper (II). Adsorption studies were conducted using 2.5 g/L biosorbent concentration in a reaction volume of 100 mL 1mM copper (II) solution. After one hour reaction time, the adsorbent was separated from the adsorbate solution and was resuspended in the desorbing solution. The desorbing agents used in the present study were 0.1 M HCl, 0.1 M EDTA, 0.1 M $CaCl_2$ solution and a distilled water solution whose pH is adjusted to 4.0 using acetate buffer. The sorbent dose was kept constant at 25 g/L and reaction time was kept as 3h.

4.2.5.7 Change in pH During Biosorption

The pH of the liquid phase after adsorption was determined. This experiment was conducted employing sorbates such as copper (II), lanthanum (III), and mixed rare earths. Hundred mL of 1 mM adsorbate solution was kept in a beaker on a magnetic stirrer. The glass electrode of a pH meter was immersed in this and the initial pH was noted. Adsorbent was added (2.5 g/L) to this and the mixture agitated by magnetic stirrer. The pH was noted continuously using a digital pH meter (Systronics, Ahmedabad, India). A blank was run in which the reaction mixture consisted of triple distilled water and biosorbent but no adsorbate.

4.2.5.8 Interruption Test

Multiple interruption test was conducted in batch reactors to understand the rate limiting step in adsorption process. Two reaction bottles containing 100 mL each of adsorbate solution (1 mM) and 2.5 g/L of adsorbent was mounted on the rotary

shaker (30 rpm). One of the bottles was taken out at intervals 5, 10, 15, 30, 60 and 120 min from the shaker, the adsorbent separated from the adsorbate solution for one hour and the adsorbent was resuspended after this time. Samples of adsorbate solution were also withdrawn at the above timings and also at 180 min from both reaction bottles and were analysed for residual copper (II).

4.2.5.9 Quantification of Calcium Exchange

Experiments were designed to evaluate calcium ions associated with the biosorbent. The calcium leaching in two types of aqueous media was tried, one employing 1 N HCl and the other triple distilled water, where pH is adjusted to 4.0 using acetate buffer. The reaction mixtures consisted of 100 mL of either 1 N HCl or triple distilled water (pH = 4.0) and 250 mg of biosorbent M_1. After agitating the mixture at 30 rpm for 3 h in a rotary shaker, the biosorbent was separated and supernatant was analyzed for calcium using EDTA as per Standard Methods (1989).

4.3 INSTRUMENTAL METHODS

4.3.1 Electron Microscope Examinations

4.3.1.1 Transmission Electron Microscope (TEM)

The transmission electron micrographs of the adsorbent before and after adsorption can reveal the location where the element has migrated during transfer from liquid to solid phase. TEM pictures of the biosorbent were taken before and after adsorption of copper onto it using a Phillips 410 LS (Phillips, The Netherlands) Transmission Electron Microscope. The following procedure was followed for sample preparation for TEM (Hayat, 1980).

Adsorption Step: Two samples were prepared, one the raw sorbent and the other on which copper was adsorbed. Adsorption was conducted in a reaction bottle consisting of 100 mL of 1 mM copper solution. The sorbent concentration was maintained at 2.5 g/L. After agitating the mixture on a rotary shaker at 30 rpm for 3 h, the sorbent was separated by settling, washed with triple distilled water and was suspended in 0.1 M phosphate buffer (pH 7.2). The blank sample was also suspended in phosphate buffer before the samples were fixed using gluteraldehyde.

Fixation and Embedding: The fixation of samples for electron microscope was done using gluteraldehyde. The medium consisted of 2% para formaldehyde and 3% gluteraldehyde with the pH maintained by 0.1 M cacodylate buffer at 7.2$\pm$ 0.1. After 24 h, the adsorbents were dehydrated using multiple washings with progressively concentrated acetone (20 %, 40 % 60 %, 80% , 100% and 100%) for 20 min each. The dehydrated samples were immobilised onto epoxy matrix.

Sectioning: Blocks were trimmed and initially 1 μm thick sections were cut in an ultratome (LKB, India) using glass knives. The sections were stained with 1 % warm aqueous toludine blue for identification of the desired area from which ultrathin section was to be made. The marked area of the tissues were trimmed into a smaller pyramid and ultrathin sections (600 ^{o}A) were cut using the non serrated edge of the glass knife. The sections were mounted on a 400 mesh copper grid, pre cleaned with 1 N HCl and dried with alcohol.

TEM pictures were taken on a Phillips 410 LS (Philips, The Netherlands) Transmission Electron Microscope at an acceleration voltage of 80 KV.

4.3.1.2 Scanning Electron Microscope (SEM)

SEM studies are useful for the detection of surface characteristics of the adsorbents. In the present study, SEM of all the six adsorbents (M_1, M_2, M_3, M_4, M_N and M_S) were taken.

Samples were dehydrated by multiple washings in increasingly concentrated acetone as in the case of TEM. These samples were then fixed onto a graphite or aluminium stub (8mm ϕ) using absolute acetone, graphite paint or silver paste. These stubs were then kept in a PS-2 silver sputtering unit (International Scientific Instruments, USA) and a 40^{o}A coating of silver was applied onto it under vacuum for 15 min.

SEM pictures were taken on a JSM 840A Scanning Electron Microscope (JEOL, Japan). Pictures were taken at accelerating voltages of 5-15 KV.

4.3.2 Energy Dispersion Analysis by X-ray (EDAX) Studies

EDAX analyses were conducted using the Kevex apparatus attached to the SEM (JEOL, Japan). Sample preparation was done in the same way as above but accelerating voltage was kept constant at 15 KV, to facilitate the emission of secondary X-rays. Elemental analysis was provided by a dedicated on line computer system..

4.3.3 Electron Paramagnetic Resonance (EPR) Studies

EPR spectra of the biosorbent samples were taken before and after adsorption of copper (II) and dysprosium (III). The particle size of adsorbent for this was < 80 μm and adsorption was conducted from a 0.1 mM copper(II) and Dy (III) solution, (adsorbent concentration 2.5 g/L) to get a fine spectrum without interferences. Adsorbent was separated by settling after the reaction, washed

thoroughly with distilled water followed by alcohol and acetone. Samples were later dried at 40^{o}C for 24 h prior to taking the spectra.

EPR spectra were taken on a E-106, X band EPR spectrophotometer (Varian Associates, California, USA) on powder samples at room temperature.

4.3.4 Infrared (IR) Spectroscopy

IR spectra of biosorbent were taken of adsorbent samples which were subjected to the sequential elution procedure. Samples were prepared in KBr pellets and analyzed on a Perkin Elmer 1600 FTIR Spectrophotometer (Perkin Elmer, USA).

4.4 FIXED BED ADSORPTION STUDIES

4.4.1 Head Loss Studies

Experiments to evaluate the head loss in a fixed bed reactor was conducted using a 600 mm high bed. The column had five ports which were connected to manometers The column with details of ports is presented in Figure 4.2. The flow rates were varied from 3 $m^3/m^2/s$ to 17 $m^3/m^2/s$ and a constant head of 750 mm was maintained throughout.

4.4.2 Attrition Studies

The physical breakdown of adsorbents in a packed bed adsorption reactor by both static and dynamic head is to be evaluated. In case of excessive rupture of the adsorbent, not only head loss increases but also the advantage of reuse of bed may not be available. To find out the degree of attrition in a dynamic condition, the column employed in aforementioned section was filled with a pre-weighed amount of biosorbent which was retained in 600 μm sieve. After allowing flow through the bed at a rate of 36 $m^3/m^2/s$ (maximum flow

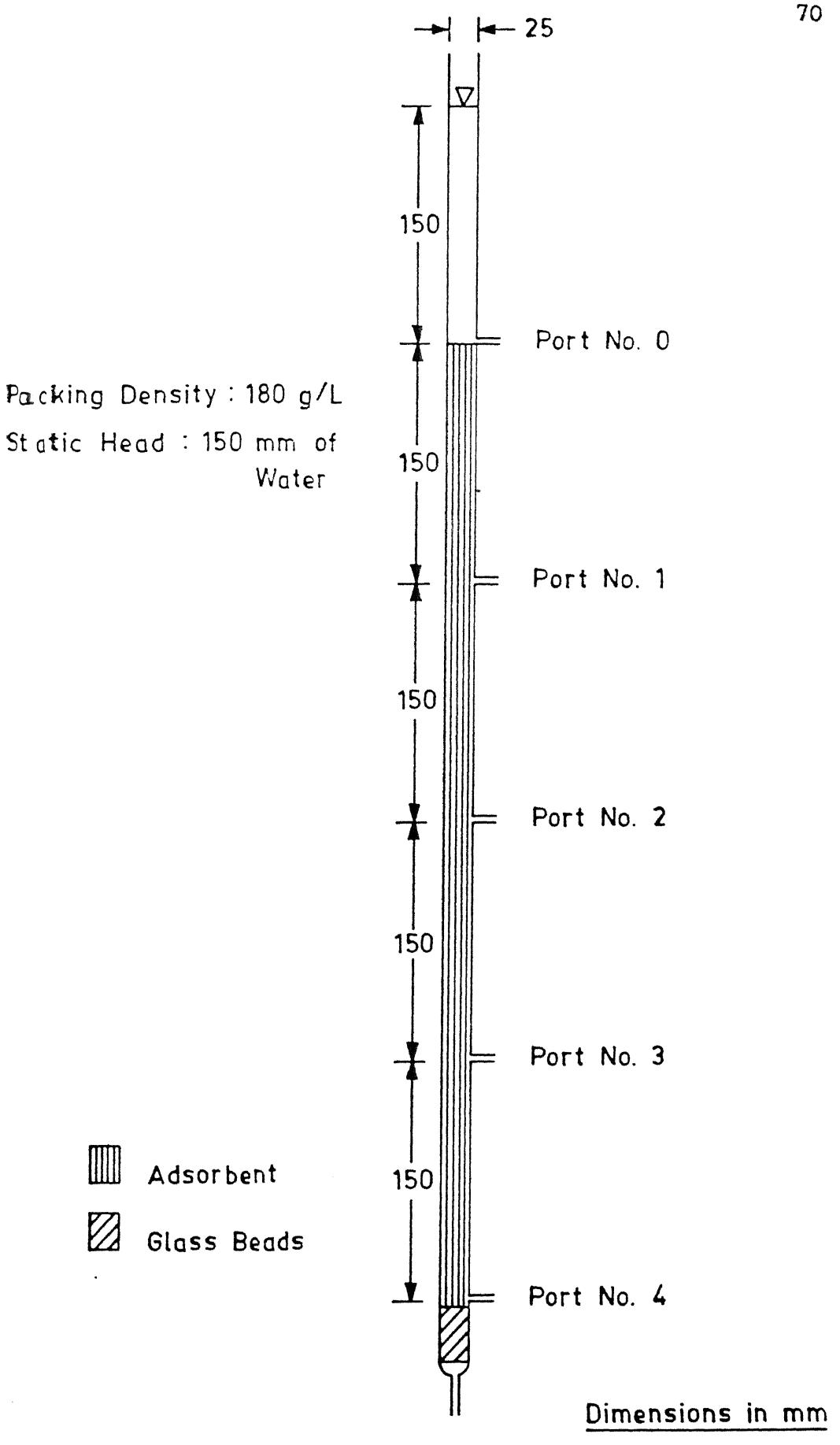


Fig. 4.2. Column Arrangement for Head loss Studies.

possible at a head of 750 mm) for 24 hours, the material was taken out, dried in an oven at 40 ^{o}C and was sieved through a 600 μm sieve. The amount of adsorbent which passed through the sieve was a measure of attrition and was reported as a percentage of the original.

4.4.3 Bench Scale Columns for Scaling-up

Adsorption experiments were conducted in a number of fixed bed reactors (down flow mode) employing mixed rare earth chloride as the adsorbate. Three columns of identical diameter of 50 mm each and lengths 300, 600 and 1200 mm were used to generate data for bed depth service time (BDST) model. The experimental setup with the flow arrangement is presented in Figure 4.3. The influent concentration was maintained constant at 250 mg/L (as RCl_3) and buffered at a pH of 4.0 using acetate buffer and the flow rate employed was 1.018 $m^3/m^2/h$. The columns were run till exhaustion of the biosorbent capacity.

4.4.4 Desorption Studies

Desorption experiments were conducted using a column of 50 mm diameter and 600 mm long column. The influent consisted of 250 mg/L (as RCl_3) rare earth chloride, buffered at pH 4.0 with acetate buffer. After the exhaustion of the column, the column was desorbed using 0.1 N HCl. After the adsorbed elements had been leached out, the column was thoroughly washed using two bed volumes of distilled water and the cycle was restarted.

4.4.5 Studies With Simulated Effluent From Monazite Processing

Effluent generated from the rare earth processing industry consists of traces of thorium, heavy metals (Cd and Zn), besides the rare earth elements. A typical effluent from rare earth

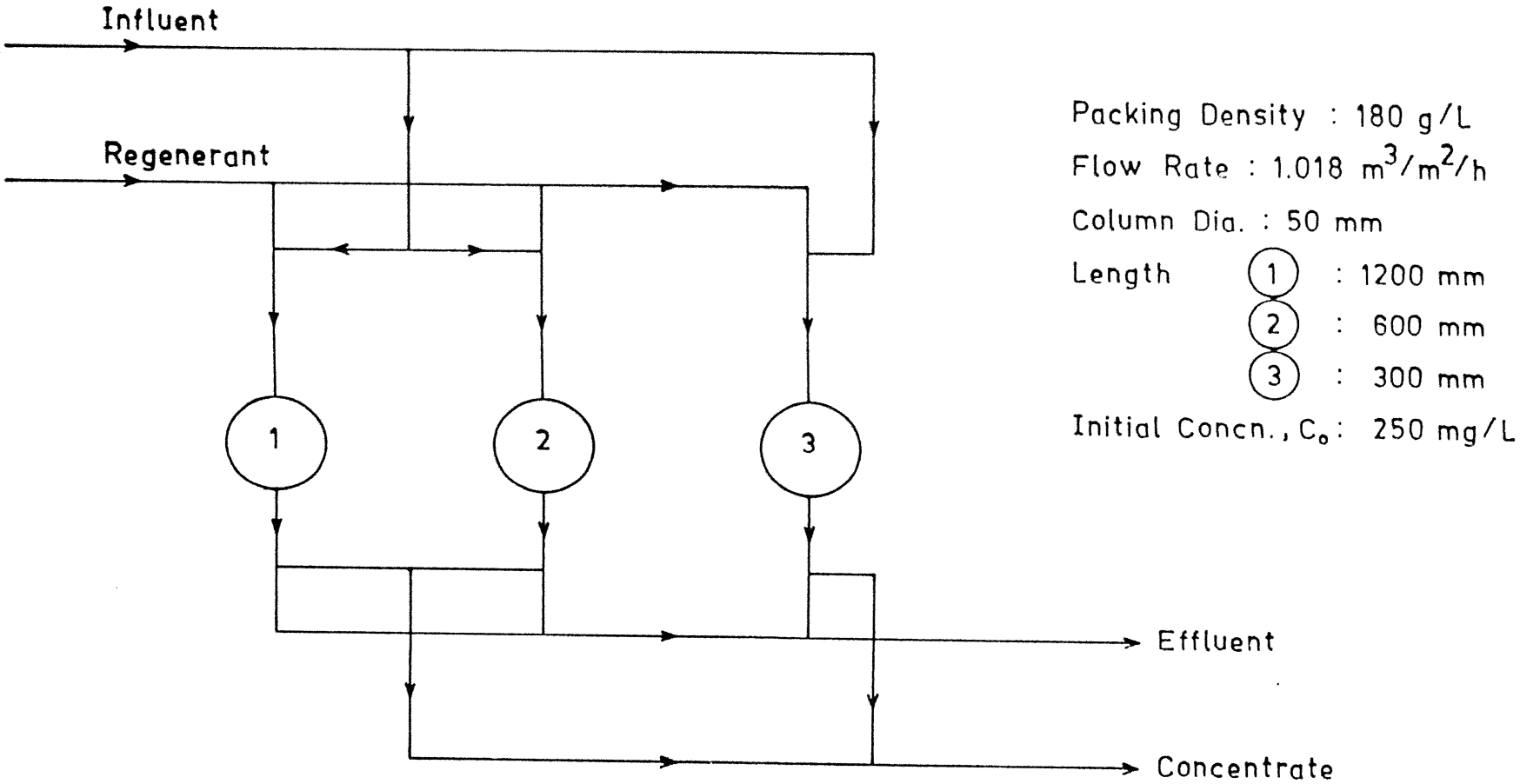


Fig. 4.3. Column Arrangement for BDST Model.

processing plant was simulated as per the composition given in Table 4.4. The effluent was passed through a column of 1750 mm length and 25 mm diameter at a rate of 1.018 $m^3/m^2/h$. The concentration of thorium and rare earths were monitored in the effluent and the column run was terminated upon exhaustion.

Table 4.4 Simulated Effluent of Monazite Processing Industries

Pollutant	Th^{4+}	RCl_3	Zn^{++}	Cd^{++}	F^-	PO_4^{3-}
Concentration (mg/L)	5	50	2	2	40	400

4.4.6 Separation of Rare Earth

A column of 25 mm diameter and 1750 mm length was packed with the biosorbent and this was challenged with a mixture of Praseodymium and Neodymium (Praseodymium and Neodymium 1g each in 3L of distilled water) at a flow rate of 1.01 $m^3/m^2/h$. The effluent was analyzed by a Shimadzu UV-Vis spectrophotometer (Shimadzu, Japan) for Nd and Pr. After the adsorption run was completed, the column was eluted with 5% citrate buffer (pH 2.53) at a flow rate of 30 mm/min. Samples were collected continuously and were analysed for Nd and Pr.

5. RESULTS AND DISCUSSION

5.1 SCOPE

In accordance with the broad objectives of the study, the results are also classified into four sections, namely, the preliminary screening of non-viable fungal species for their metal uptake potential, characterization and treatment of the selected biosorbent, delineation of metal uptake mechanism and finally field applications.

Representative results of experiments have been presented either in graphical or in tabular form. Experiments were repeated sufficient number of times to ensure statistical quality control and the values given are mean of the experimental results. Necessary standards were incorporated wherever the situation demanded.

Preliminary experiments were conducted using copper (II) as the model adsorbate. The selection of copper as the model metal ion was guided by both scientific and practical reasons such as

1. Copper is an ideal transition element to represent toxic metal as it is essential in trace concentrations and is harmful at higher concentrations to biological systems
2. Enormous information is available regarding its bioinorganic chemistry (coordination geometry, stability constants of the complexes formed) and environmental importance (toxicity, essentiality, concentration response etc.)
3. The spectral response and characteristics of copper and copper complexes have been studied extensively.

Studies have also been conducted with other metals as and when required to delineate necessary details regarding the versatility and

specificity of the biosorbent. The applicability and/or limitations of extrapolating the data obtained for copper to other elements have also been highlighted at relevant sections.

The concentrations of metals and ligands used are expressed in molar strengths for the ease of comparison. The pH of the adsorbate solution was maintained at 4.0 using o.1 M acetate buffer (2.5 mL of buffer in 50 mL adsorbate solution), unless mentioned otherwise. The adjustment of pH was done for the following reasons

1. Most metals precipitate as their hydroxides at higher pH values and conducting adsorption study at these elevated pH values is thus of limited practical importance.
2. At very low pH values, adsorption is affected drastically due to the proton competition and/or lability of the bonds. The adsorption experiments at lower pH values will therefore not reflect the metal uptake characteristics of biosorbents (Faust and Aly, 1987).
3. Most anionic ligands interfere with metal uptake to varying degrees (Tobin and others, 1987). However acetate has only low stability constant with most metals. Rao (1989) has also observed that interference of acetate exerts minimum effect on metal adsorption.

The adsorbent concentration in most experiments was maintained at 2.5 g/L, which was fixed from preliminary test results. As will be seen in following pages, while a higher concentration of adsorbent would have achieved a better percentage removal of the pollutant, it was felt that a sub-optimal concentration would reflect better the

effect of various environmental parameters on the adsorbent performance.

5.2 PRELIMINARY SCREENING OF FRUITING BODIES OF MACROFUNGI FOR BIOSORPTION POTENTIAL

As obvious from the literature review, biosorption has emerged as a promising and viable alternative metal recovery technology. However due to the incomplete understanding of the mechanism of biosorption and consequent absence of any rational method for *a priori* prediction of the biosorption potential of any microorganism, the only method for identifying and developing newer and efficient biosorbents is the sustained screening of microbes.

The presence of extensive biodiversity available in tropical forests has been identified as the treasure box for the emerging field of biotechnology (Anonymous, 1986). Many species of commercial interest in other areas of biotechnology (Agriculture, Industrial fermentation, Pharmaceuticals) were identified in the vast genetic pool of tropical forests (Newmark, 1983). It was therefore, considered appropriate to conduct an exploratory search for the presence of potential biosorbents in the tropical forests. Because of the vast number of species available, the scope of the search was demonstrative rather than comprehensive.

The study involved the screening of fruiting bodies of macrofungi for their metal uptake capacity for subsequent use as biosorbents. Fungal fruiting bodies (Mushrooms) were considered ideal for the purpose of evaluation as biosorbents because

1. Many species of the phylum fungi have been demonstrated to possess excellent biosorptive potential (Muzzareli and others,

1980; Tsezos and Volesky, 1981; and Zhou and Kiff, 1991) and mushrooms also belong to the same phylum.

2. Mushrooms grow prolifically and are found in most parts of the world (Smith, 1963). Cultivation of mushrooms is also a highly developed industry, the world wide demand for cultivated mushrooms being of the order of 3.5 million ton with an estimated economic value of $ 7 billion (Chang, 1987). Collection, preservation and marketing of naturally growing mushrooms is a well established activity in many parts of the world (Shad and Lakhanpal, 1991). Mushrooms can thus be grown in a laboratory or on an industrial scale, should a need arise. The availability of mushrooms, either from natural or from commercial units hence will not be rate limiting for adoption of the process. Further, nonedible mushrooms have no competing commercial use and hence they could be procured economically.

3. Mushrooms are macro in size, tough in texture and have other physical characteristics which are conducive for their development into adsorbents, without the need for immobilization or deployment of sophisticated reactor configuration as in the case of microorganisms.

Though there have not been many reported studies on utilization of fungal fruiting bodies as biosorbents, their potential for heavy metal uptake is not totally unknown. Much effort has been spent in the last decade to investigate the heavy metal accumulation in higher fungi of the edible variety, mostly because of the possibility of their entry into humans via terrestrial food chain (Brunnert and Zadrazil, 1983)

Mushrooms are the fruiting bodies of fungi and are organized to produce spores. In the present study, the fruiting bodies of nine fungal species were evaluated. All species were collected from tropical forests/plantations. The criteria for selection of these species were.

1. Earlier studies on the bioconcentration of metals by edible fungal species indicated that though there are remarkable differences between species in their ability to concentrate metals, in general metal uptake capacity of saphrophytic species were better than other species (Lepsova and Mejstrik, 1988). Because of this, only saphrophytic organisms were evaluated for their metal uptake potential.
2. Only those species which were not considered as edible were evaluated. This was done to avoid the competing use and consequent unfavorable economics at the stage of field use.
3. Many fungal fruiting bodies are degraded rapidly upon exposure to natural environment and/or physical forces. Since the study envisaged developing an adsorbent which will have to be in contact with water for most of the time and also will have to withstand high water pressures, only those species which possessed apparent stability (resistance to wilting off in rainy season) and toughness (resistance to crushing) were collected.

The saturation uptake capacity of the biosorbent is the parameter generally used to compare the potential of different organisms (Macaskie and Dean, 1990). The preliminary screening of the mushroom species were done by conducting equilibrium uptake studies. The

saturation uptake potential was thereafter calculated by appropriate mathematical analysis of the data.

5.2.1 Equilibrium studies

When an adsorbent is contacted with an adsorbate, an apparent equilibrium is established between the adsorbate concentration in the liquid phase and that on the solid phase. This state is one of dynamic stability, ie., the amount of adsorbate migrating onto the adsorbent is counterbalanced by the amount of adsorbate migrating back into the liquid phase and should not be construed as a state of cessation of adsorption. The adsorption equilibria can hence be modeled along the lines of a heterogeneous chemical reaction (Barrow, 1979).

If it is assumed that the uptake of metals by biosorbents is the result of a chemical coordination between the metal and a chemical moiety on the adsorbent, it follows that the maximum number of such sites available are finite. Let it be denoted by Q_{max}. Now the equilibrium state can be described analogous to the definition of gas adsorption as suggested by Langmuir (1918). The Langmuir theory assumes a monolayer of adsorbates over the adsorbent, no interaction between adjacent adsorbate molecules once adsorbed onto the adsorbent and also assumes that all sites of adsorption are energetically equivalent.

If C_e is the equilibrium concentration of adsorbate in the liquid phase, q, the number of sites on adsorbent occupied by adsorbate and θ, the fractional saturation of the sites of interaction on the adsorbent, then Langmuir theory suggests that the rate of desorption from the

adsorbent surface can be taken to be proportional to the fractional saturation, θ

ie., Rate of desorption = $k_1 \theta$ 5.1

The rate of adsorption however is assumed to be proportional to both the amount of sites available on the adsorbent and the concentration of adsorbate in the liquid phase (C_e)

ie., Rate of adsorption = $k_2 C_e (1-\theta)$ 5.2

where k_1 and k_2 are proportionality constants,

At equilibrium,

Rate of adsorption = Rate of desorption

$$k_2 C_e (1-\theta) = k_1 \theta \quad 5.3$$

Rearranging

$$\theta = \frac{k_2 C_e}{k_1 + k_2 C_e} \quad 5.4$$

introducing a constant $b = k_2/k_1$

$$\theta = \frac{b C_e}{1 + b C_e} \quad 5.5$$

If Q_{max} is the maximum adsorption site available on the sorbent and q_e, the equilibrium coverage (number of sites occupied) at an aqueous phase concentration C_e

then $\theta = q_e/Q_{max}$ 5.6

$$q_e = \frac{Q_{max} b C_e}{1 + b C_e} \quad 5.7$$

The maximum uptake capacity Q_{max} can be evaluated from Eq. 5.7 after conducting a series of adsorption experiments. Equilibria experiments were conducted using all nine of the selected adsorbents with initial adsorbate concentration varying from 0.2 to 2 mM of copper (II). The equilibrium sorption curves are given in Figure 5.1 to 5.9. All equilibrium distributions follow the typical saturation profile.

The equilibrium distribution curve can be linearised in many ways for the evaluation of Q_{max}, the saturation adsorption capacity. A direct linearisation results in a plot similar to the classical Lineweaver-Burke plot

$$\frac{1}{q_e} = \frac{1}{Q_{max}} + \frac{1}{Q_{max} b C_e} \qquad 5.8$$

Another form of linearisation can be obtained by multiplying both sides by C_e

$$\frac{C_e}{q_e} = \frac{C_e}{Q_{max}} + \frac{1}{b Q_{max}} \qquad 5.9$$

Multiplying equation 5.8 by $q_e\ Q_{max}$ and rearranging, we can get yet another linearised form

$$q_e = Q_{max} - \frac{q_e}{b C_e} \qquad 5.10$$

Any one of these linearised forms can be used for the evaluation of the saturation capacity Q_{max} and the constant b.

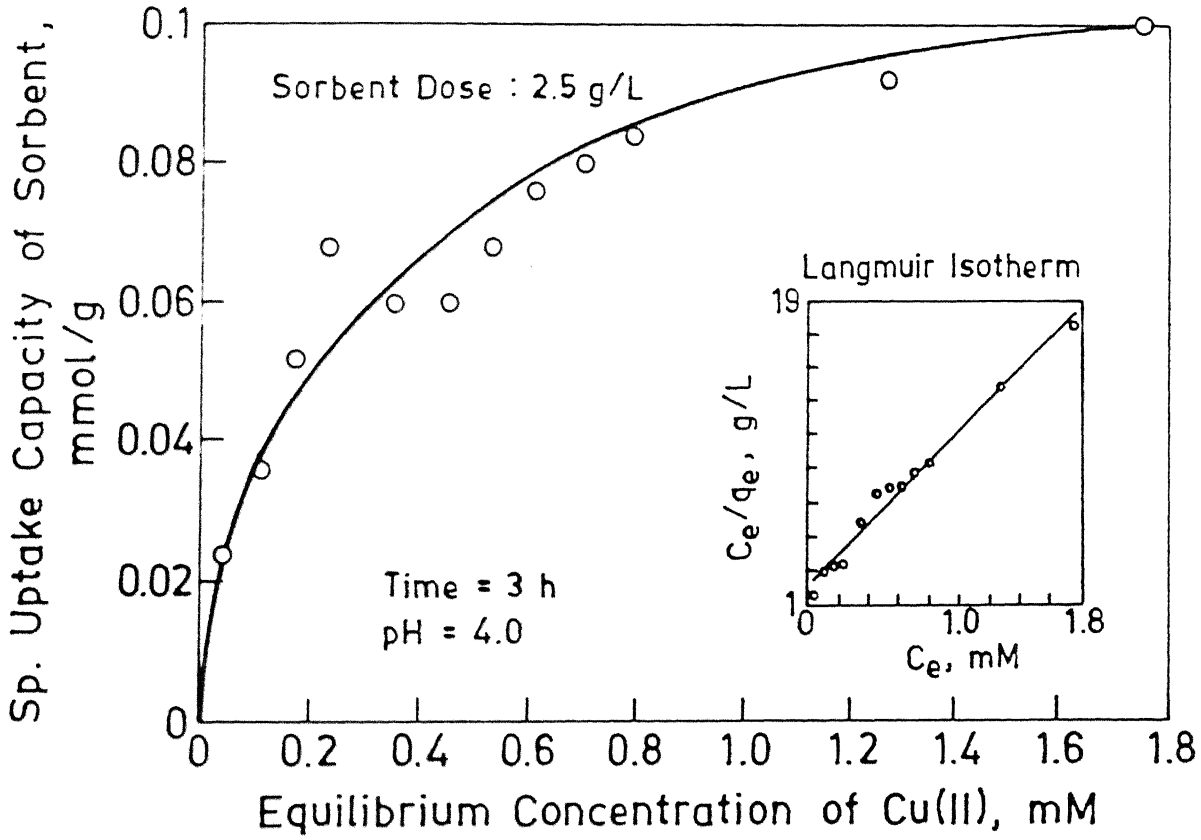


Fig. 5.1. Equilibrium Sorption Curve for Cu(II) by Coriolopsis strumosa.

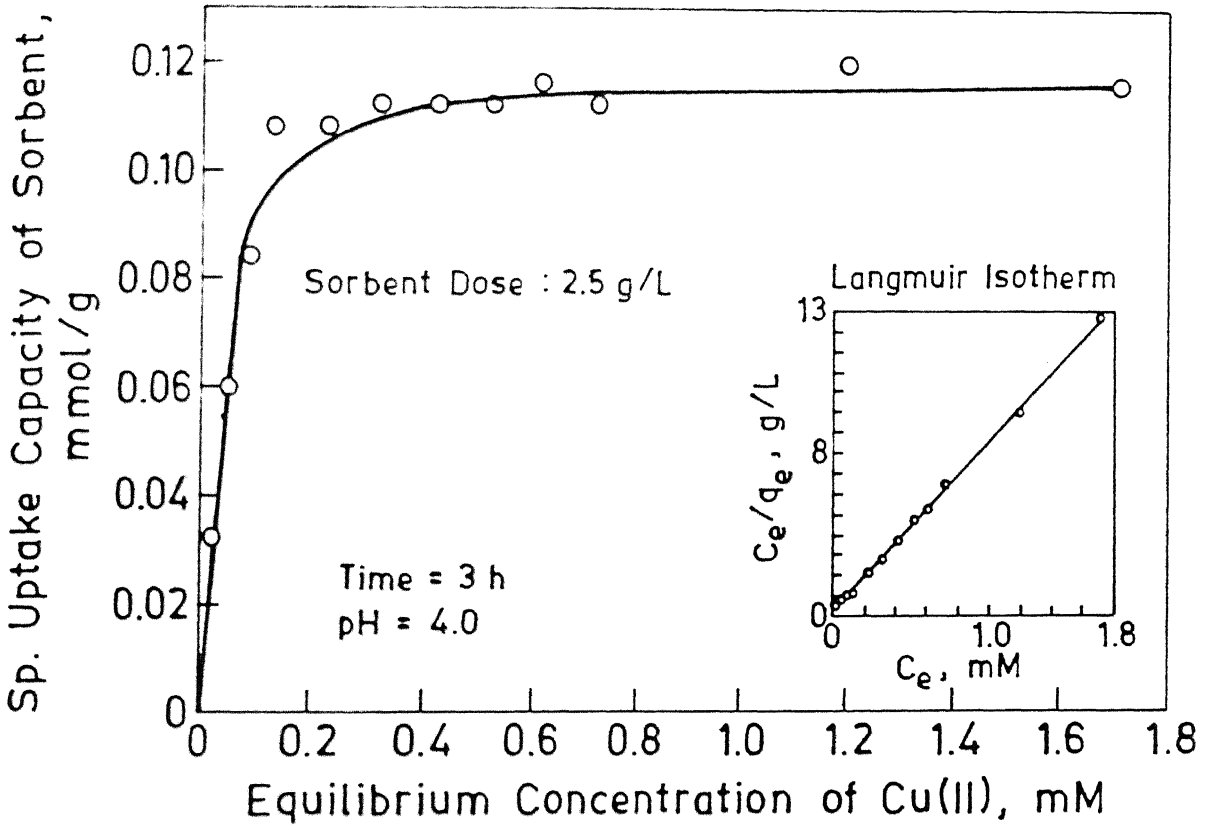


Fig. 5.2. Euilibrium Sorption Curve for Cu(II) by Daedalea tenuis.

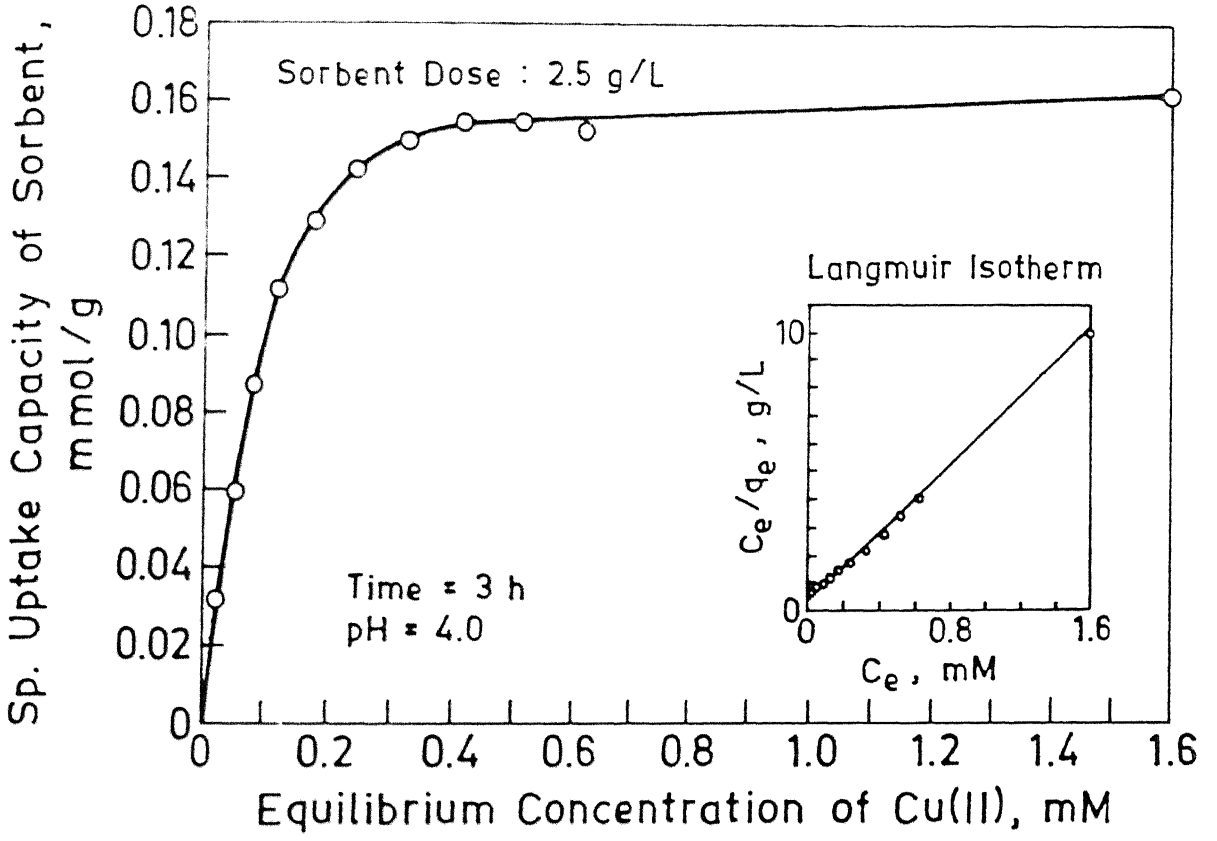


Fig. 5.3. Equilibrium Sorption Curve for Cu(II) by Lentinus strigosus.

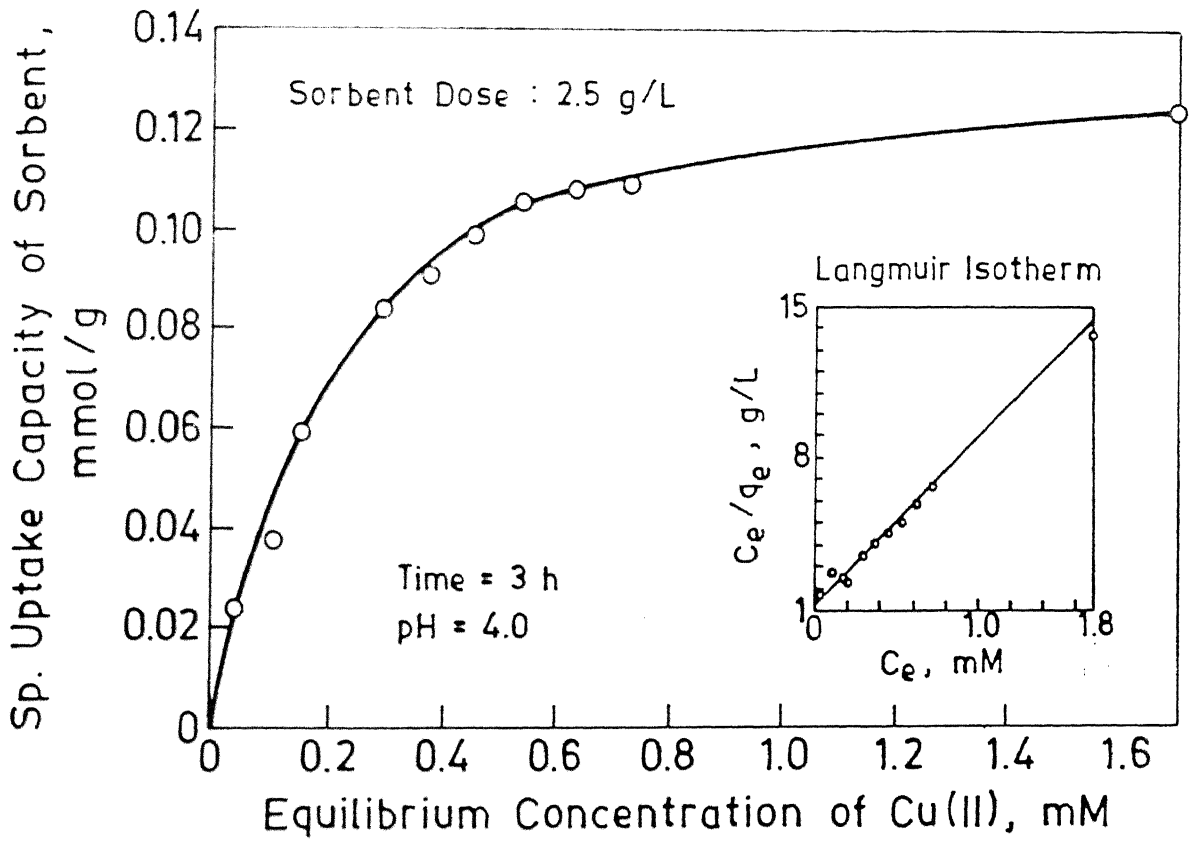


Fig. 5.4. Equilibrium Sorption Curve for Cu(II) by Lenzites malaccenis.

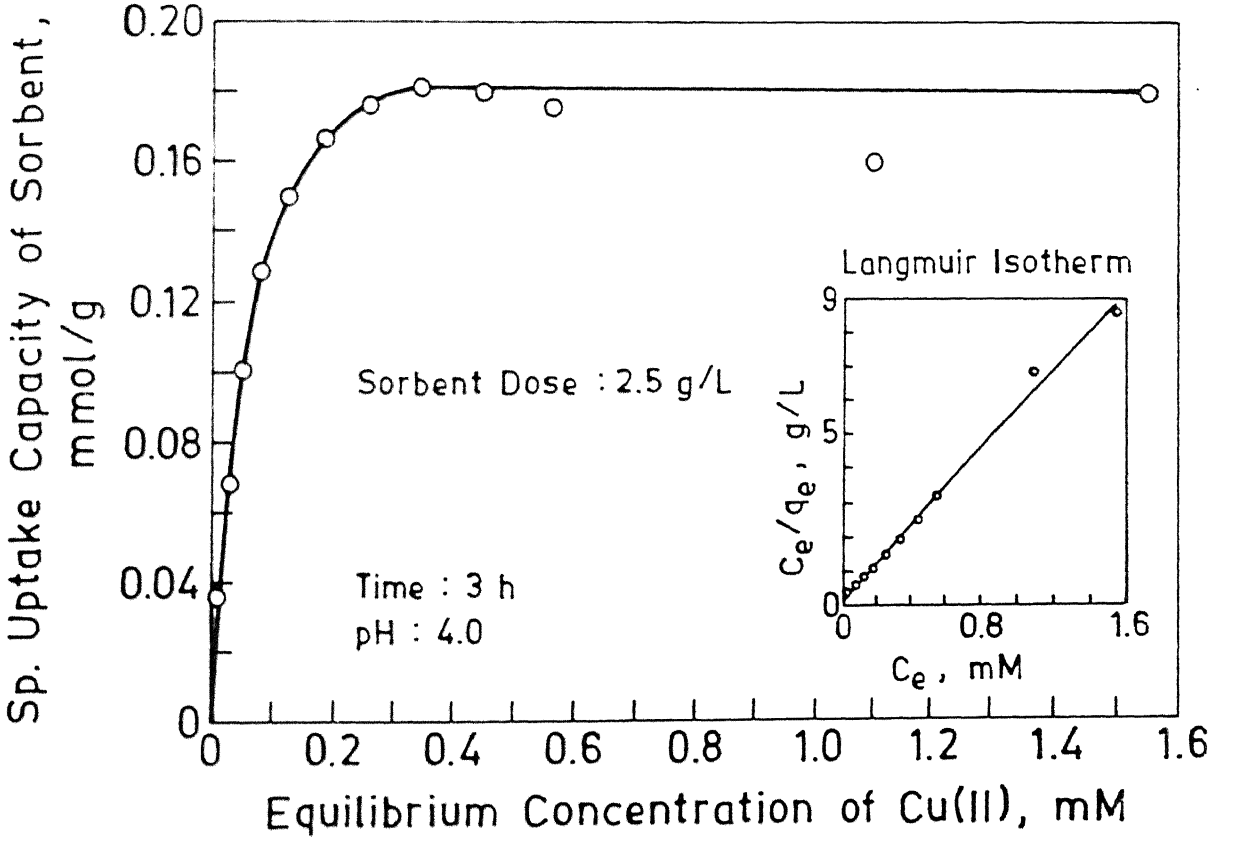


Fig. 5.5. Equilibrium Sorption Curve for Cu(II) by Phellinus xeranticus.

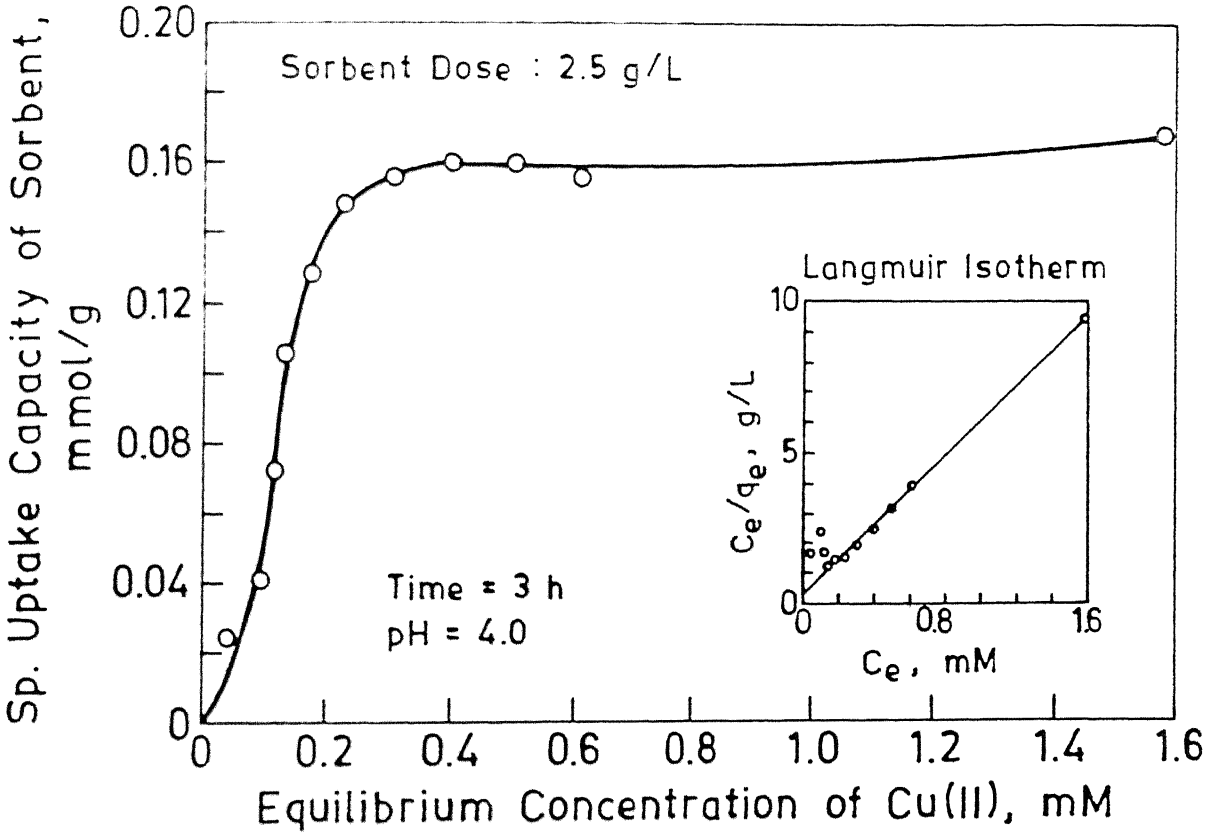


Fig. 5.6. Equilibrium Sorption Curve for Cu(II) by Rigidoporus lineatus.

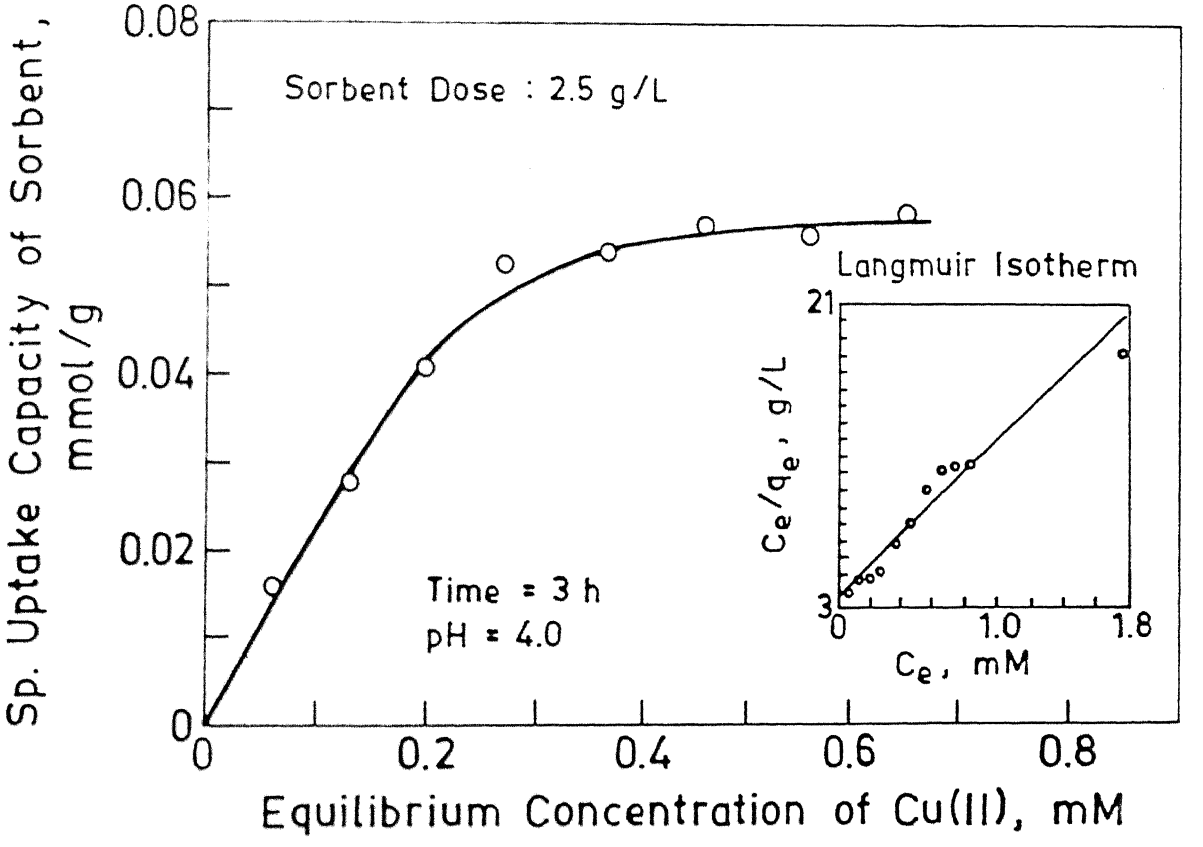


Fig. 5.7 Equilibrium Sorption Curve for Cu(II) by Rigidoporus microporus.

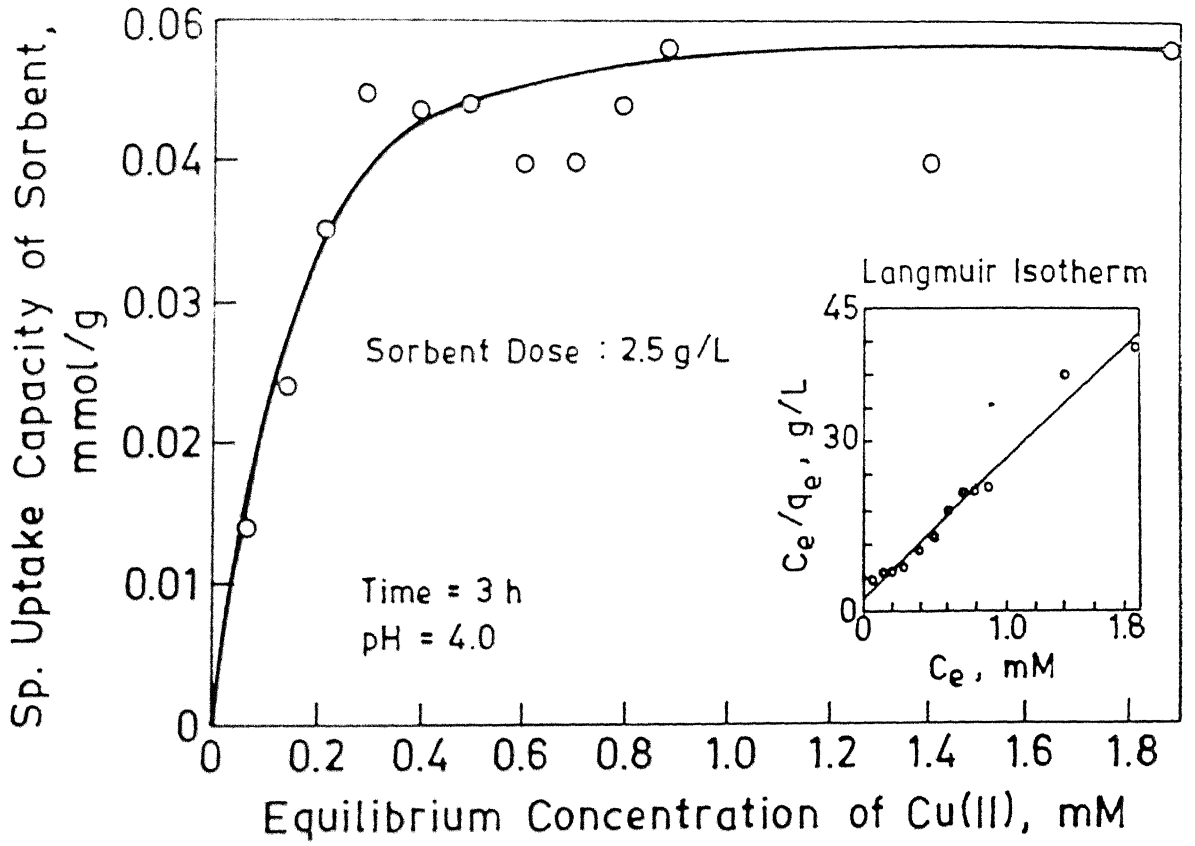


Fig. 5.8. Equilibrium Sorption Curve for Cu(II) by Trametes lactenia .

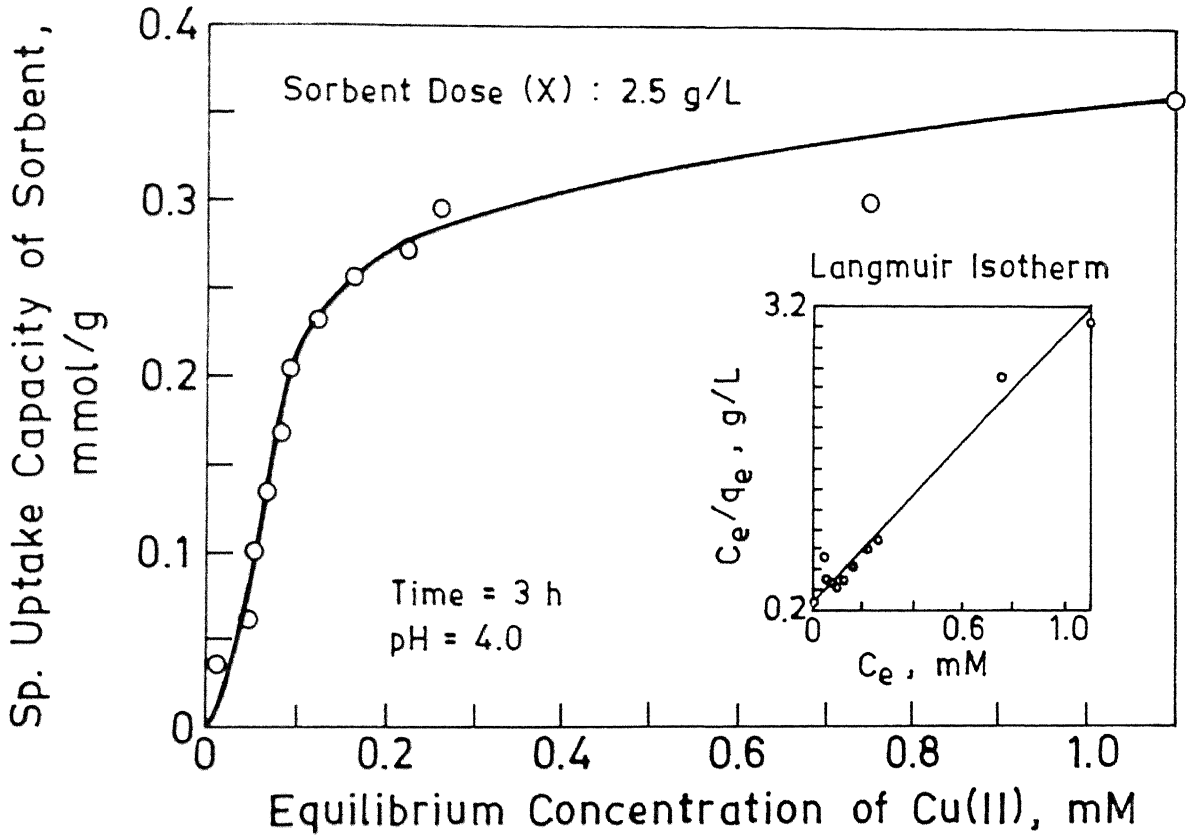


Fig. 5.9. Equilibrium Sorption Curve for Cu(II) by Ganoderma lucidum (Biosorbent M_1).

If the adsorption data were to follow an ideal mathematical pattern, the values of Q_{max} and b would have been the same whatever be the linearisation used. However due to the divergence of experimental data from the idealized format, evaluation of the constants will result in different values depending upon the linearisation followed. Since equation 5.8 involves two reciprocal quantities prone to experimental errors, it generally results in a lower coefficient of correlation than the other two linearisations (Rubin and Mercer, 1981). In such cases, the values of the constants are evaluated by all the possible linearisations and that value which resulted in the best correlation coefficient is reported (Bhattacharya, 1983). In the present work, the constants were evaluated by all the three linearisations and the value which gave maximum coefficient of correlation between the independent and dependant variable is reported.

In the present study, equation 5.9 resulted in a better linearisation for all cases and this is presented in the insets of Figure 5.1 to 5.9. Table 5.1 gives the tabulation of Q_{max} values for different adsorbents.

It can be seen from the results that all species adsorb metals but there is considerable variation in the extent of metal uptake. *Trametes lactenia* took up only 0.048 mmol/g copper whereas *Ganoderma lucidum* took up 0.383 mmol/g under identical conditions, the other species having uptake values in between.

It will be relevant here to compare the metal uptake potential of the mushroom species studied with those adsorbents reported in literature. A brief compilation of the metal uptake potential of some widely reported biosorbents is presented in Table 5.2. Even field

Table 5.1 Maximum Metal Uptake Capacity (Q_{max}) of Different Fungal Species

Species	Uptake Capacity Q_{max},(mmol/g)
Trametes lactenia	0.048
Rigidoporus microporus	0.108
Coriolopsis Strumosa	0.113
Daedalea tenuis	0.120
Lenzites malaccenis	0.130
Rigidoporus lineatus	0.163
Lentinus Strigosus	0.164
Phellinus xeranticus	0.178
Ganoderma lucidum	0.383

Table 5.2 Copper (II) Uptake Capacity of Some Reported Biosorbents

Species	Uptake Capacity Q_{max} (mmol/g)	Reference
Activated Sludge	0.125	Rao and Venkobachar (1989)
Aspergillus niger	0.120	Muzzareli and others (1980)
Cladosporium resinae	0.120	Rome and Gadd (1987)
Penicillium italicum	0.150	ibid
Penicillium spinulosum	0.040	Townsley and others (1986)
Rhizopus arrhizus	0.250	Tobin and others
Filtrasorb 400	0.030	Present Study

scale units have been developed usi some of these as biosorbents indicating their commercial viability. *Ganoderma lucidum* exhibited a metal uptake capacity far exceeding all these reported biosorbents. Among nine mushroom species investigated in the present study, eight of them have exhibited metal uptake capacity more than 0.1 mmol/g which is comparable to that by other reported biosorbents. All species of mushroom performed better than Filtrasorb 400 (0.030 mmol/g) which is generally employed for heavy metal removal.

There exists a positive potential for developing promising biosorbents from fruiting bodies of macrofungi available in tropical forests. Their capacity is comparable to those of other reported biosorbents and far exceeds that of Filtrasorb 400. The rationale behind the screening is thus vindicated.

Metal uptake potential of microbial species has often been described in relation with their toxicity to these elements. Toxic metals may be accumulated by the bacteria as a part of their de-toxification strategy. It is therefore interesting to note here that *Ganoderma lucidum* which gave the best metal removal potential has also been reported to be highly resistant to copper fungicides (Menon, 1963). Toxicity relationship regarding other species were however not available and hence a meaningful comparison could not be attempted.

Though the study identified a number of biosorbents with potential to be economically developed into effective biosorbents, further studies were restricted to the detailed evaluation of the species which gave the best uptake values. Thus *Ganoderma lucidum* which gave outstanding metal uptake potential was used for further

studies. The subsequent sections deal with development of a pretreatment strategy and characterization of the adsorbent.

5.3 PRETREATMENT OF BIOSORBENT *G. lucidum* AND ITS EVALUATION

Many biosorbents do not exhibit their full potential in the raw form and their metal uptake capacity has been found to improve dramatically upon chemical pretreatment (Muzzareli and others, 1980; Brierly and others, 1986; Townsley and others, 1986). Pretreatments have also been found to be necessary because many micro organisms which possess good biosorption potential are often found not to be ideally suited for field application. This is because, in addition to metal uptake potential, an adsorbent should also possess characteristics suitable for its employment in an adsorption reactor. Though many pretreatment methodologies have been suggested and evaluated for improving the metal uptake potential of biosorbents, no rational basis for these chemical treatments has been spelt out. As a result, it is difficult to appreciate the scientific basis rendering the improvement brought about by such treatments. These observations are often empirical in nature. Further, most reports are concerned with only the apparent increase in the metal uptake potential of the biosorbent upon pretreatment without reporting the changes in the physico-chemical characteristics of the adsorbent material, which may be also of significance as far as the practical utility is concerned.

In the present study, a series of chemical pretreatments were given to the biosorbent *G. lucidum*. While some treatments were aimed at changing the chemical nature of the biosorbent, others were

directed to improve the stability of sorbent to leaching. The biosorbents derived after subjecting *G. lucidum* to various treatments were then evaluated for their metal uptake potential, rate of metal uptake and were also subjected to relevant physico-chemical characterization to register the changes that have occurred.

Five derivatives were prepared from the biosorbent *G. lucidum*. The biosorbent without any pretreatment is designated as M_1. A second derivative was obtained by treating the biosorbent M_1 with a mixture of methanol and chloroform, which is designated as M_2. A third derivative was produced by treating the biosorbent M_2 by cold 2N NaOH, this is designated as M_3. Another product was obtained by treating biosorbent M_3 with 40 % NaOH at 120 ^{o}C and is designated as M_4. Two of the products produced by formaldehyde treatment of the biosorbent M_1 in the presence of nitric acid and sulphuric acid are referred as M_N and M_S respectively.

Leaching of intracellular components into the aqueous medium is one of the problems associated with the utilisation of biosorbent and formaldehyde treatment is reported to reduce the leachability characteristics of adsorbents (Freer and others, 1989).

5.3.1 Equilibrium Copper (II) Uptake Studies

The different biosorbent derivatives prepared from *G. lucidum* were subjected to equilibrium metal uptake studies. The sorbent concentrations were kept constant at 2.5 g/L and sorbate concentrations were varied from 0.2 to 2 mM. All studies were conducted at a constant pH value of 4.0, maintained using acetate buffer. The results of equilibrium studies conducted with these adsorbents are presented in Figure 5.10 to 5.14 and the best

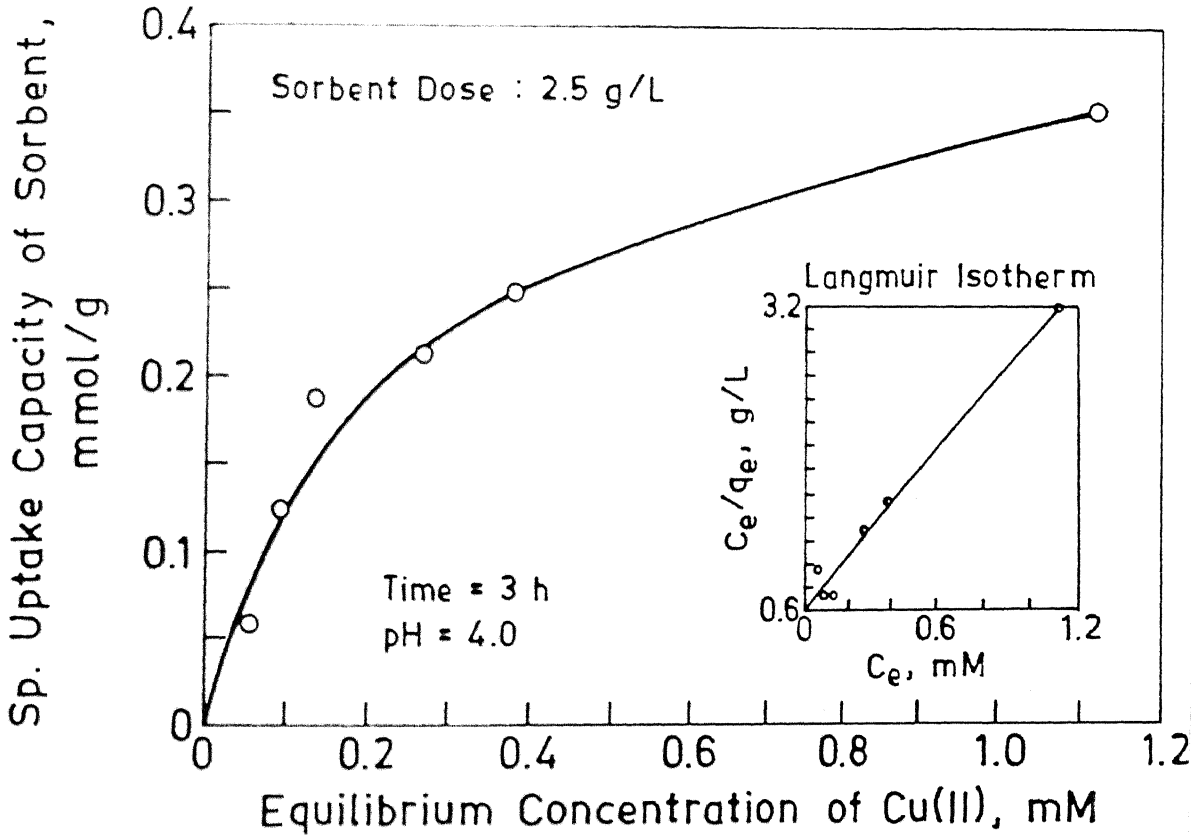


Fig. 5.10. Equilibrium Sorption Curve for Cu(II) by Biosorbent M_2.

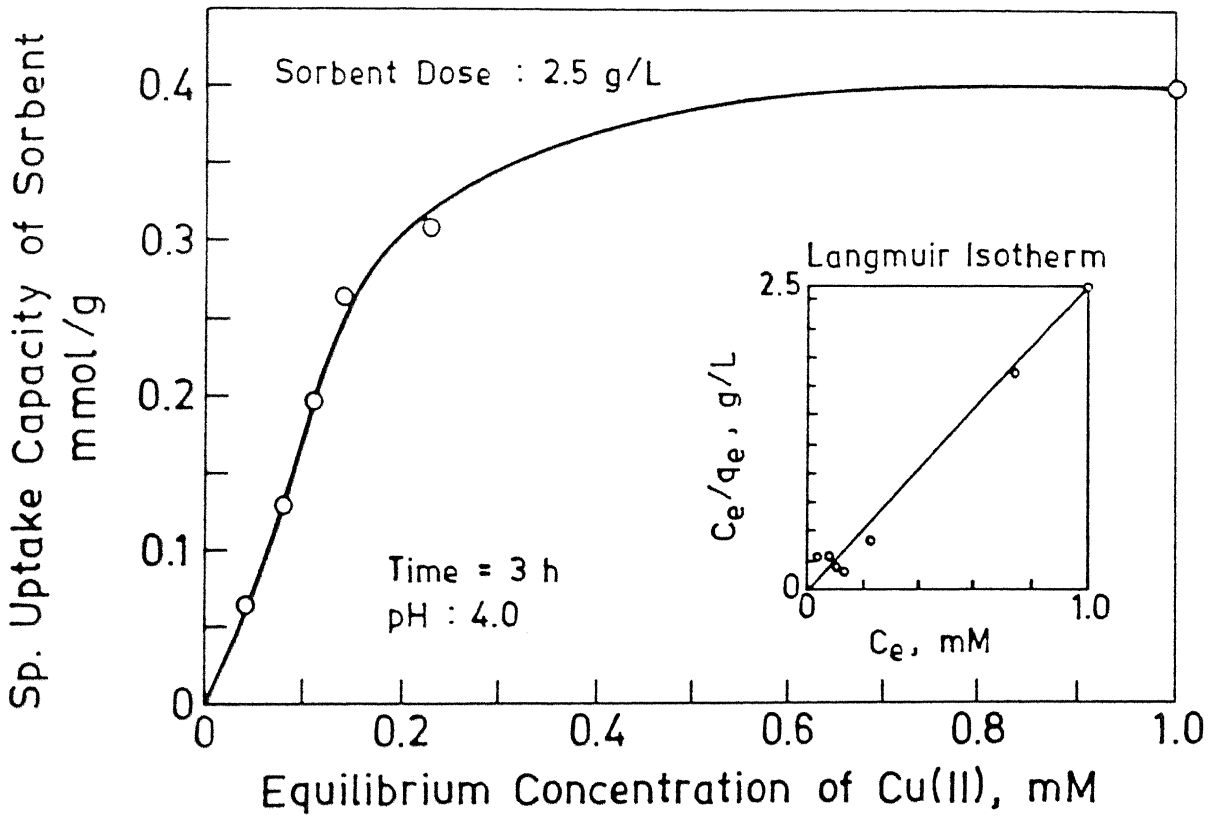


Fig. 5.12. Equilibrium Sorption Curve for Cu(II) by Biosorbent M_4.

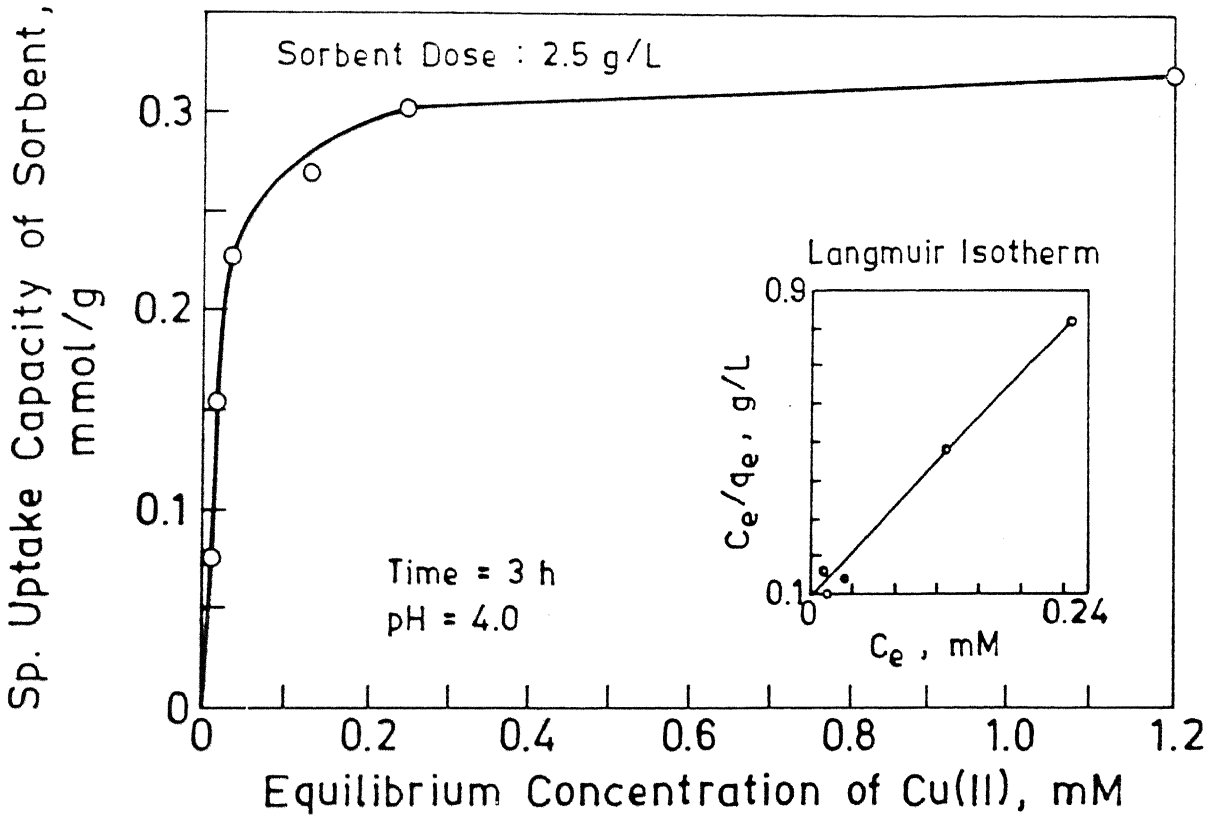


Fig. 5.13. Equilibrium Sorption Curve for Cu(II) by Biosorbent M_N.

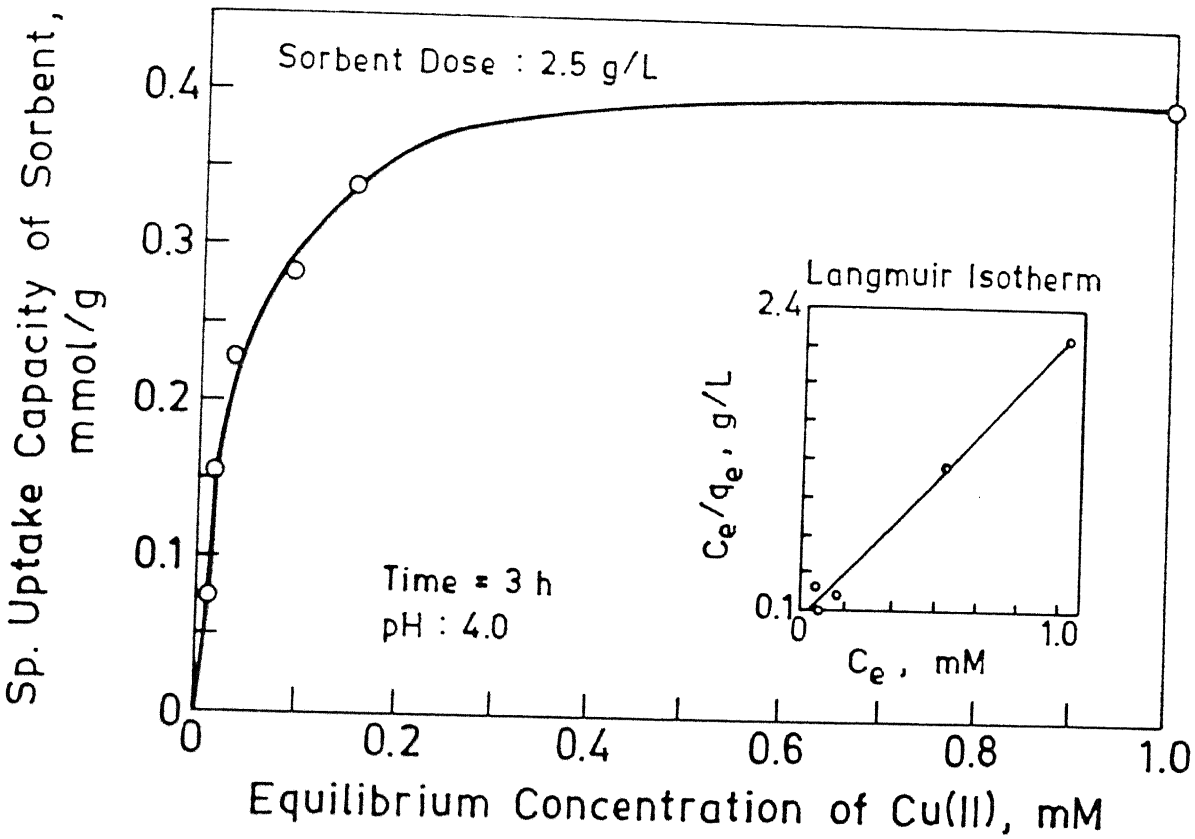


Fig. 5.14. Equilibrium Sorption Curve for Cu(II) by Biosobent M_S.

linearisations of Langmuir isotherm as per preceding sections are depicted in the insets. However, the equilibrium sorption curve for M_1 (*Ganoderma lucidum*) is presented in Figure 5.9. The saturation uptake potential of different biosorbent derivatives is presented in Table 5.3. The apparent Q_{max} values vary from 0.33 mmol/g for M_N to 0.478 mmol/g for M_4, the value for untreated biosorbent (M_1) being 0.383 mmol/g. There is however another factor to be accounted for, ie., the weight reduction of the biosorbent consequent to chemical treatments. The yield of individual biosorbents (M_2 to M_S) as a fraction of the weight of M_1 and the uptake capacity corrected for the weight loss is presented in Table 5.3.

Table 5.3 Apparent and Actual Metal Uptake Capacity of Different Biosorbents

Biosorbent	Uptake Capacity Apparent (mmol/g) Q_{max}	Yield Y	Uptake Capacity Actual(mmol/g) $Q_{max} * Y$
M_1	0.383	1.00	0.383
M_2	0.436	0.96	0.398
M_3	0.451	0.82	0.370
M_4	0.478	0.65	0.312
M_N	0.425	0.79	0.337
M_S	0.332	0.84	0.283

When the uptakes are brought down to a common datum, it can be seen that there is no actual increase in the metal uptake. The apparent increase in metal uptake capacity is, however, significant from a practical point of view. To treat the same volume of copper bearing effluent, a reactor employing M_4 need to be only three fourth as that of a reactor employing M_1.

It can be observed from the above experiments that the biosorbent G. lucidum does not require any pretreatment to achieve its maximum metal uptake potential. This is a significant advantage against other reported biosorbents.

5.3.2 Kinetics of Copper (II) Uptake by Biosorbents

In addition to the uptake capacity, the rate of uptake of the adsorbate by the biosorbent is also critical as far as the reactor configuration is concerned. A rapid kinetics will facilitate smaller reactors (lower contact time for effective adsorbate transfer) where as a slow rate of uptake will necessitate long columns or series of columns to utilize maximum potential of the adsorbent.

Rate of adsorption is usually measured by determining the change in concentration of the adsorbate in contact with the adsorbent as a function of time. Many investigators have proposed linearisation of this adsorption data by plotting the amount of adsorbate adsorbed per unit weight of the adsorbent (q_e) versus square root of time ($t^{1/2}$) (Weber and Morris, 1963; Zogorski, 1975). The slope of this curve, designated as adsorption rate, has been employed for comparison of different adsorbents. It is to be understood, however, that these

adsorption rates (unit mmol/g/$h^{1/2}$ or equivalent) are not true reaction rates but relative ones useful for comparison only.

The kinetic profile of biosorption data for the sorbents employed is presented in Figure 5.15 - 5.20. The linearisation according to the above described method is presented in the insets. As can be observed, the experimental results follow a linear profile for first fifteen minutes, whereafter it deviates. Such a trend has also been observed by other workers (Bhattacharya and Venkobachar, 1984) while studying cadmium removal onto many low cost adsorbents. In such cases it was suggested that the initial linear portion of the curve could be utilized for calculation of removal rate (Prasad and Venkobachar, 1988). A compilation of adsorption rates calculated based on the linear profile of first fifteen minutes, for the six adsorbents is presented in Table 5.4.

Table 5. 4 Rate Constant for Different Biosorbent Derivatives

Sorbent	Rate Constant $_{1/2}$ mmol/g/min
M_1	0.0620
M_2	0.060
M_3	0.061
M_4	0.061
M_S	0.070
M_N	0.070

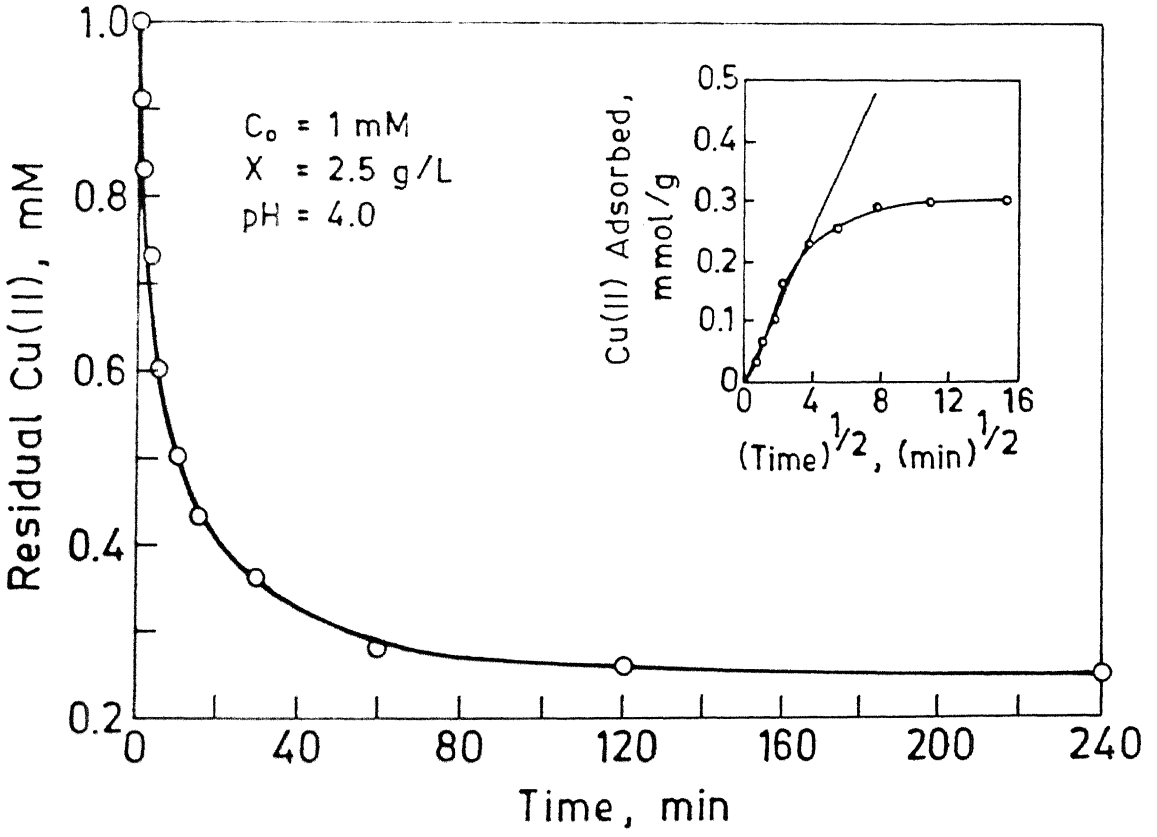


Fig. 5.15. Kinetics of Biosorption of Cu(II) by Biosorbent M_1.

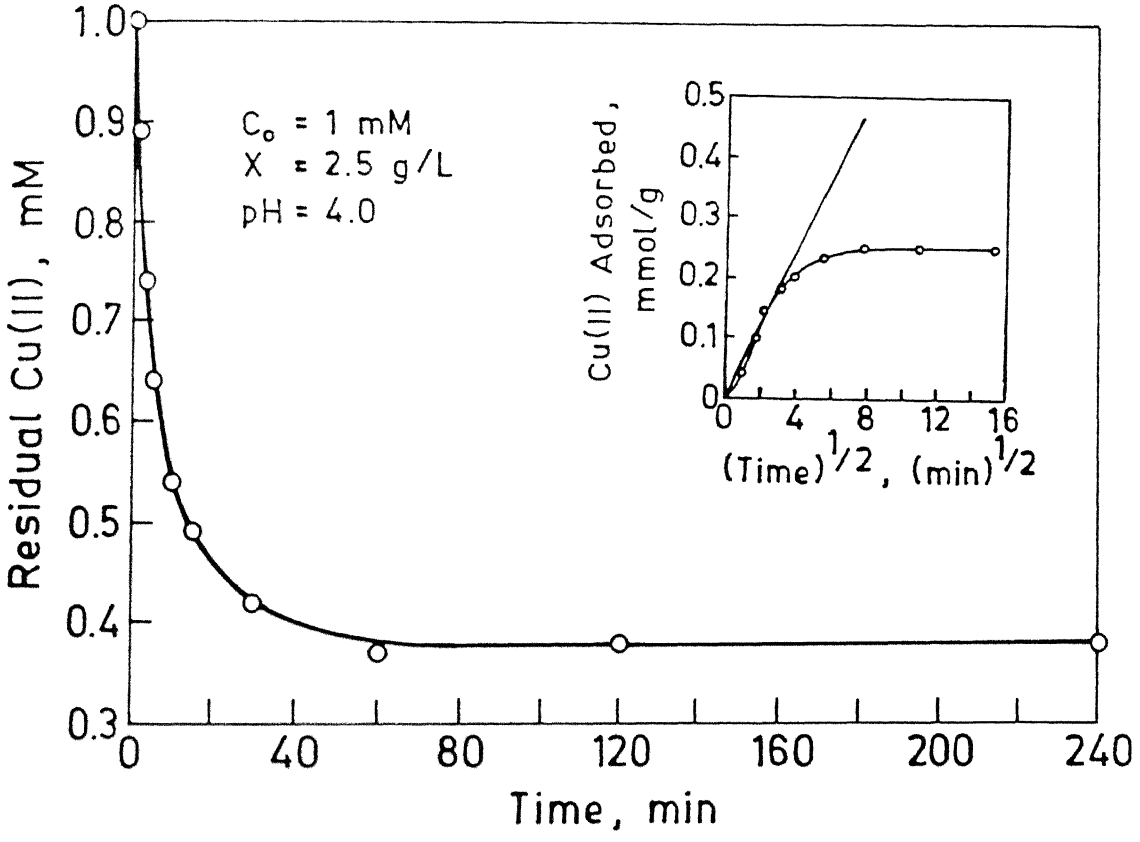


Fig. 5.16. Kinetics of Biosorption of Cu(II) by Biosorbent M_2.

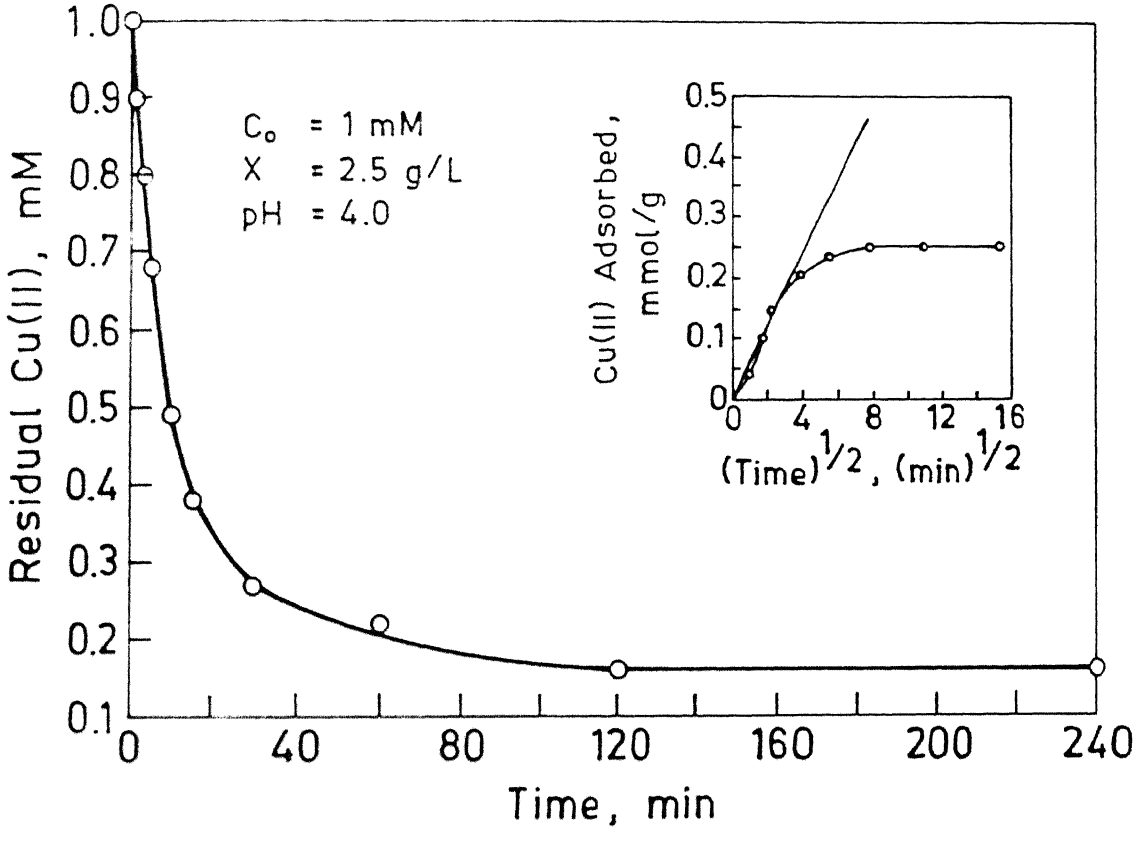


Fig. 5.17. Kinetics of Biosorption of Cu(II) by Biosorbent M_3.

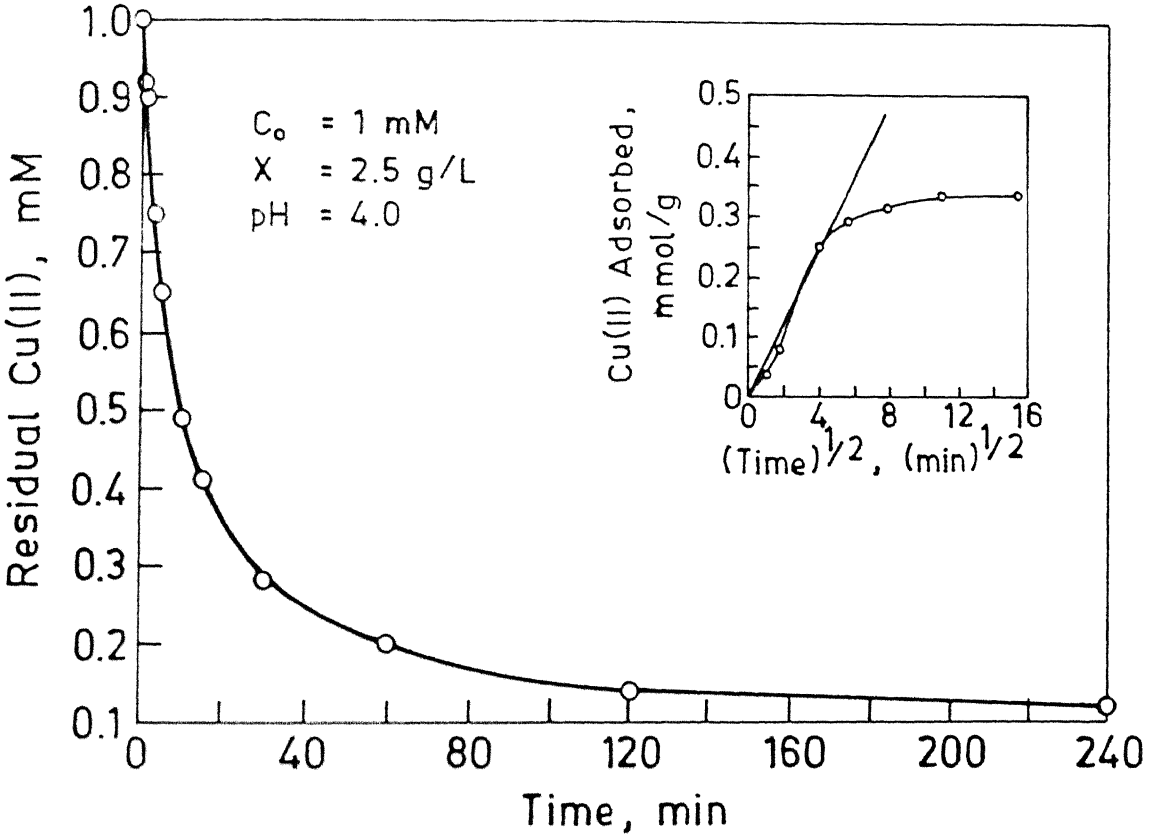


Fig. 5.18. Kinetics of Biosorption of Cu(II) by Biosorbent M_4.

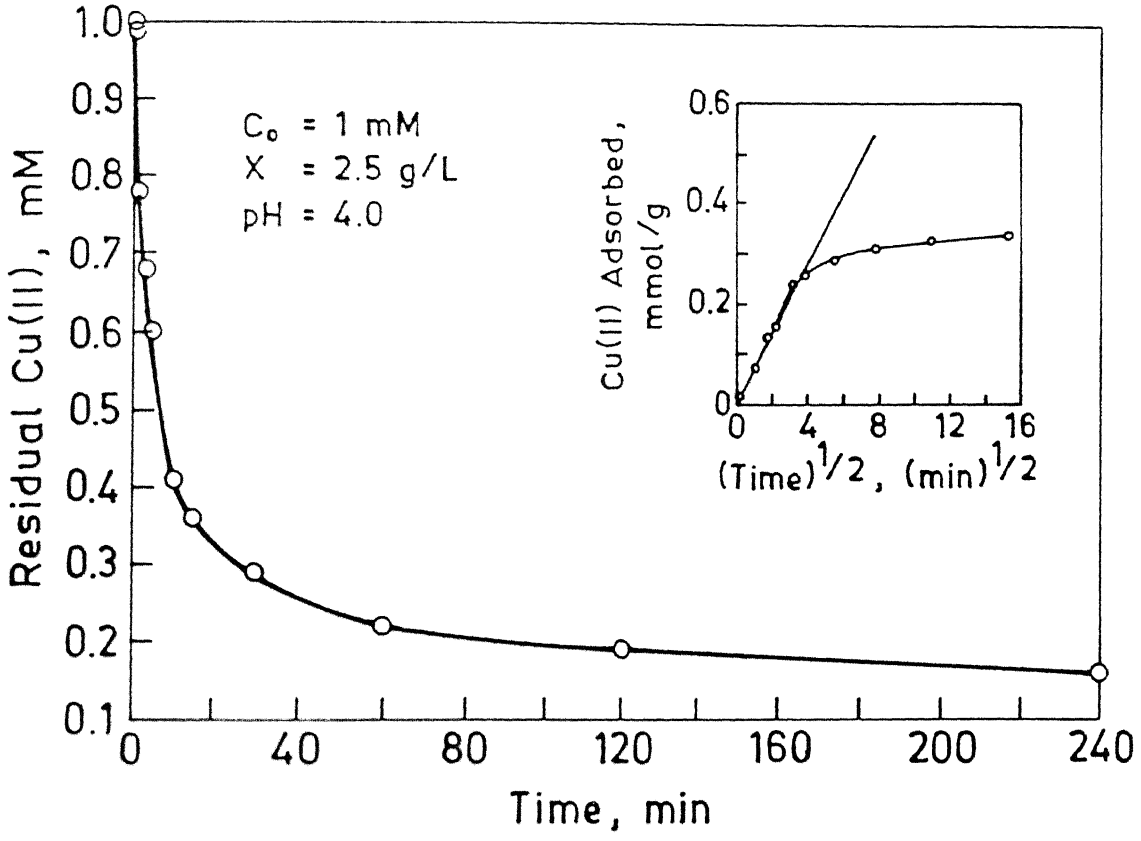


Fig. 5.19. Kinetics of Biosorption of Cu(II) by Biosorbent M_S.

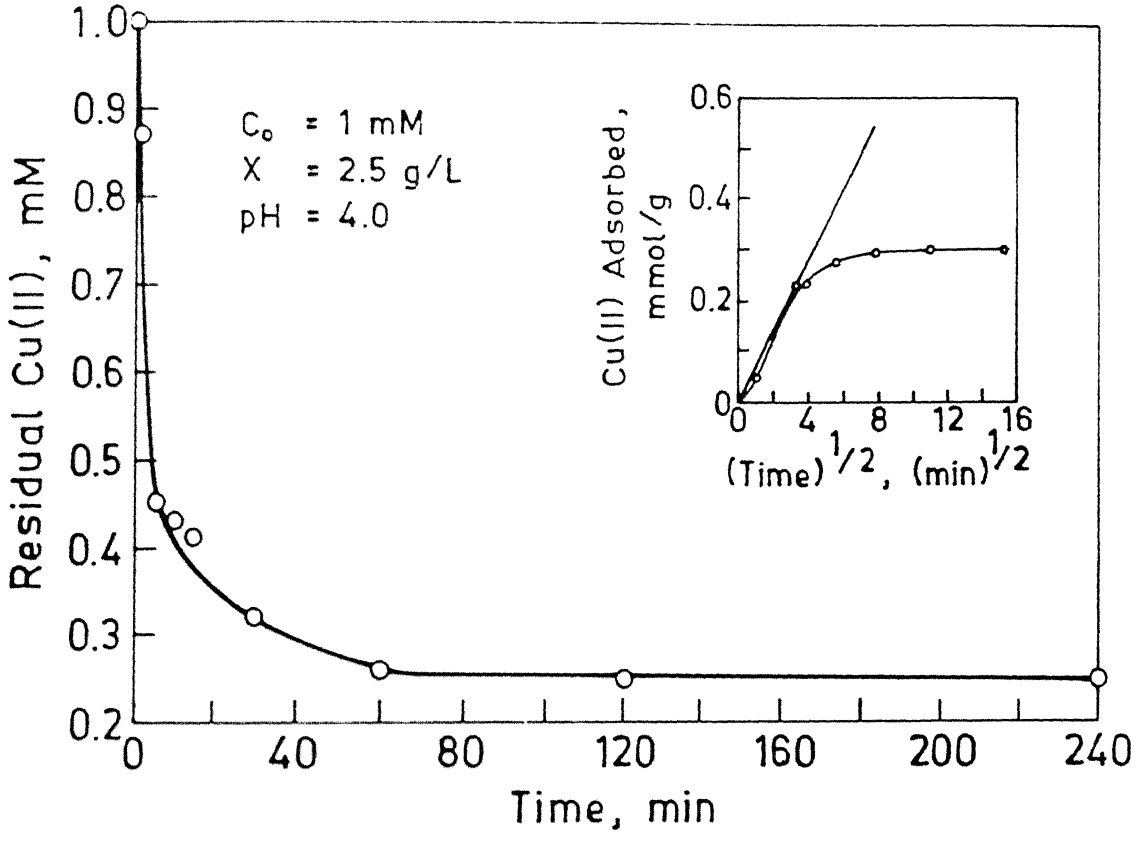


Fig. 5.20. Kinetics of Biosorption of Cu(II) by Biosorbent Mn.

The various derivatives prepared from biosorbent *Ganoderma lucidum* have nearly equal rate constants indicating that the pretreatments have not substantially changed the properties of the biosorbent from a kinetic point of view. The rate constant values are at least one order of magnitude greater than the rate constants values reported by Bhattacharya and Venkobachar (1984) for cadmium removal onto low cost adsorbents. This rapid kinetics has significant practical importance as it will facilitate smaller reactor volumes ensuring efficiency and economy.

5.3.3 Characterization of Adsorbent

The specific metal uptake capacity and the kinetics of biosorption have been the two primary deciding tests for evaluating and comparing the biosorbents. However, these two parameters alone do not represent the practical viability of the biosorbent. Many biosorbents which exhibited favorable metal uptake capacity and rapid kinetics did not exhibit excellent performance when they were employed in reactors. The problems encountered include difficulty in solid liquid separation, chemical and thermal degradation, mechanical destruction, and development of high head loss in packed bed reactors (Huber and others, 1990; Sharma, 1990).

All reported applications of biosorbents required its immobilization on some stable matrix (Tsezos, 1990) or granulation of microbes (Brierly and others, 1986). Microbial biomass in its native form consists of extremely small particles of low density, low mechanical strength and low rigidity. The use of such materials in any conventional unit operation for treating effluents containing metal ions is not practicable due to difficulty in achieving rapid and

efficient separation of biomass from the reaction mixture after contact (Brierly and others, 1986; Krambeer, 1987). To obviate this difficulty, immobilized microbial systems are employed. Immobilization, however, is not a sure-fire solution. In addition to the cost involved, it may cause detrimental alterations to the biosorbents in terms of both metal uptake and kinetic behavior (Tsezos, 1990)

In view of the observed difficulties encountered in employing biosorbents, which performed excellently in the laboratory, during scale up, it was felt necessary that the comparison be done more comprehensively taking into account the physico-chemical characteristics of the adsorbents. Hence the objective was to evaluate the physical properties of the biosorbents, the chemical and thermal stability in addition to their behavior in a packed bed reactor. No standard criteria have however been proposed as yet for the evaluation of the physico-chemical properties of biosorbents. The testing method for ion exchangers and adsorbents as suggested by American Water Works Association (AWWA, 1943) was therefore taken as a guideline and the methods were modified to suit biosorbents. Experimental protocol was designed taking into account the likely adverse environments in a realtime situation.

The different parameters evaluated and values obtained are presented in Table 5.5.

5.3.3.1 Stability to Chemical Attack

As clear from the data presented in Table 5.3, the biosorbent *G. lucidum* retains a substantial part of its weight as well as metal uptake capacity even after subjecting it to drastic

Table 5.5 Characterisation of Biosorbents

Parameter	Biosorbent					
	M_1	M_2	M_3	M_4	M_N	M_S
Apparent Density (g/l)	100	114	163	189	155	152
Specific Gravity	1.06	1.10	1.10	1.15	1.10	1.10
Ash Content (%)	2.07	5.52	6.5	6.8	6.2	6.2
pH	6.58	6.82	7.28	7.50	7.14	7.16
Survival at 180^{o}C (%)	All > 95 %					
% Change in Uptake	All within 5%					
Survival at 1.089 Kg/Cm^2 and 121^{o}C	All > 95%					
% Change in Uptake	All within 5%					
Leaching as COD (mg/L)	40	56	72	48	40	16
Apparent Density (Wet) (g/l)	181	195	202	220	190	192
Filterability ($m^3/m^2/h$)	36	38	42	42	40	41
Head Loss at 3.5 $m^3/m^2/h$ in cm/cm	All less than 1/60					
Hardness (as % Survival)	86.40	87.12	85.32	77.16	85.27	84.17
Water Soluble Matter (%)	0.50	0.25	0.20	0.20	0.30	0.30

chemical treatments, both acidic and alkaline. In the field, it is highly unlikely that the biosorbent will encounter an alkaline solution equivalent to 40 % NaOH. Similar is the case with concentrated acid (3% HNO_3). In both the above cases (employed in the process of preparation of derivatives M_4 and M_N respectively) the chemical treatments did not alter the metal uptake capacity or kinetics substantially. It can therefore be easily concluded that the biosorbent possess chemical stability suitable for application within the pH range likely to be encountered in normal field conditions.

5.3.3.2 Scanning Electron Microscope Studies

Scanning electron microscope (SEM) studies of the biosorbents were conducted to understand the physical nature of the biosorbent surface as well to investigate the physical damage to the surface, if any, due to drastic chemical treatments. The SEM pictures of biosorbents M_1, M_2, M_3, M_4, M_S, and M_N are presented in Figure 5.21 to 5.26. The pictures clearly indicate that the biosorbents are of highly porous in nature, which is a highly desirable characteristic for any adsorbent. It can also be seen that the drastic treatments have not brought about any substantial visible change in the biosorbent, further confirming that the biosorbent *G. lucidum* possess an excellent resistance to chemical attack. Other reported scanning electron microscope studies were, however, conducted with granulated biosorbents (Brierly and others, 1986; Volesky, 1990) and hence they are not representative of the biosorbing microbe itself. A comparison of the surface properties is, therefore, not attempted.

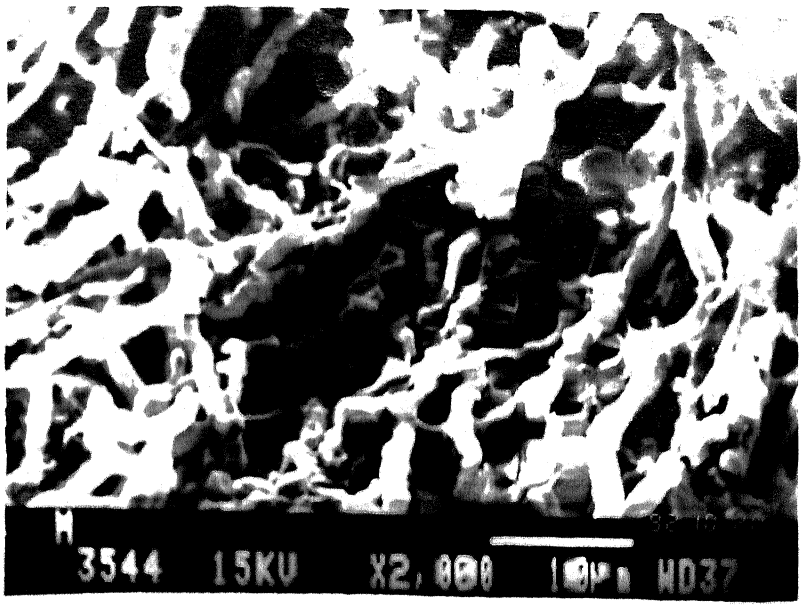


Fig. 5.21. Scanning Electron Micrograph of Biosorbent M_1.

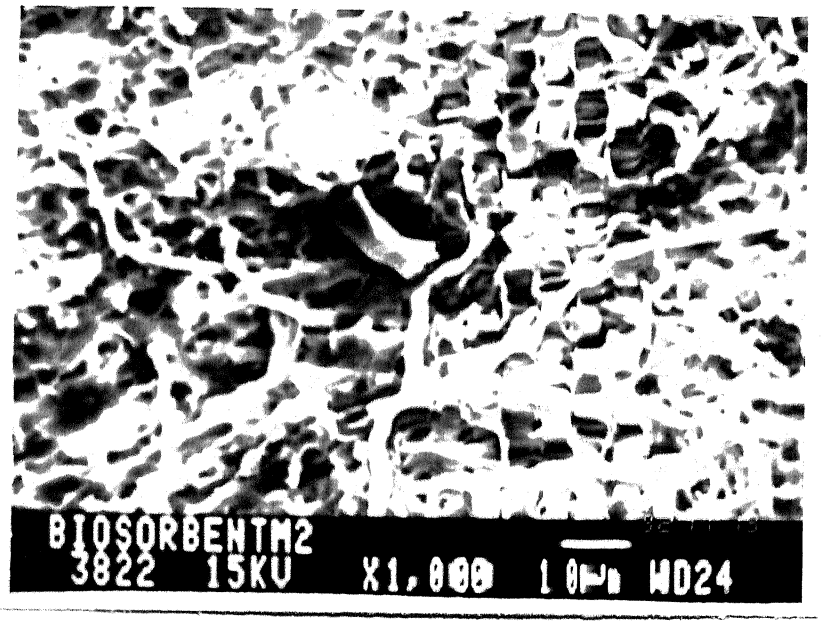


Fig. 5.22. Scanning Electron Micrograph of Biosorbent M_2.

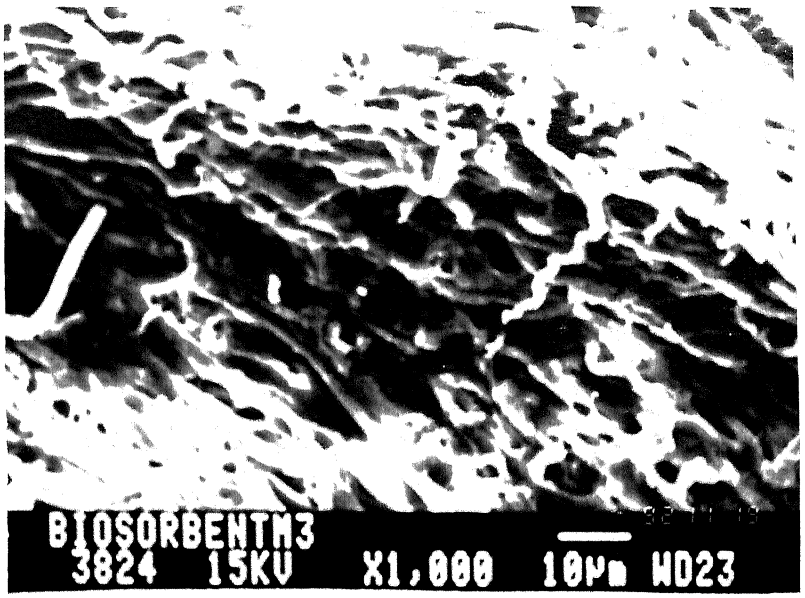


Fig. 5.23. Scanning Electron Micrograph of Biosorbent M_3.

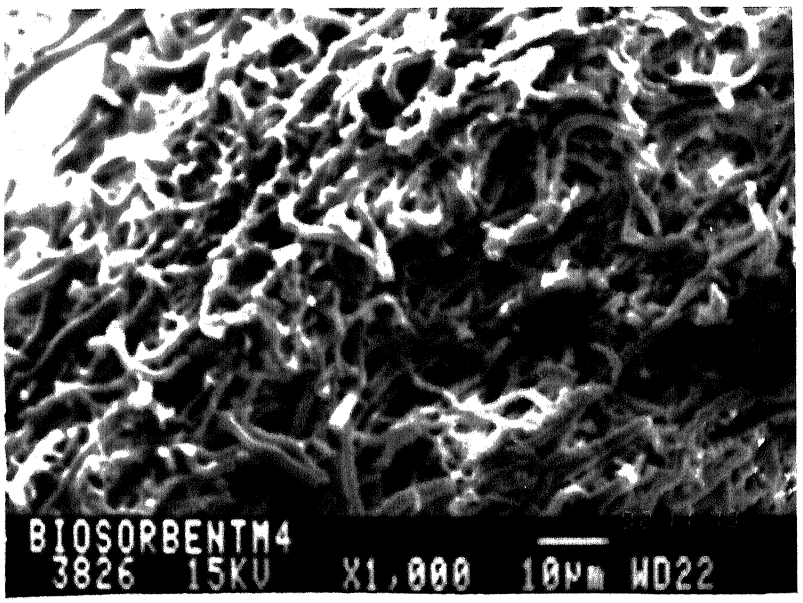


Fig. 5.24. Scanning Electron Micrograph of Biosorbent M_4.

Fig. 5.25. Scanning Electron Micrograph of Biosorbent M_S.

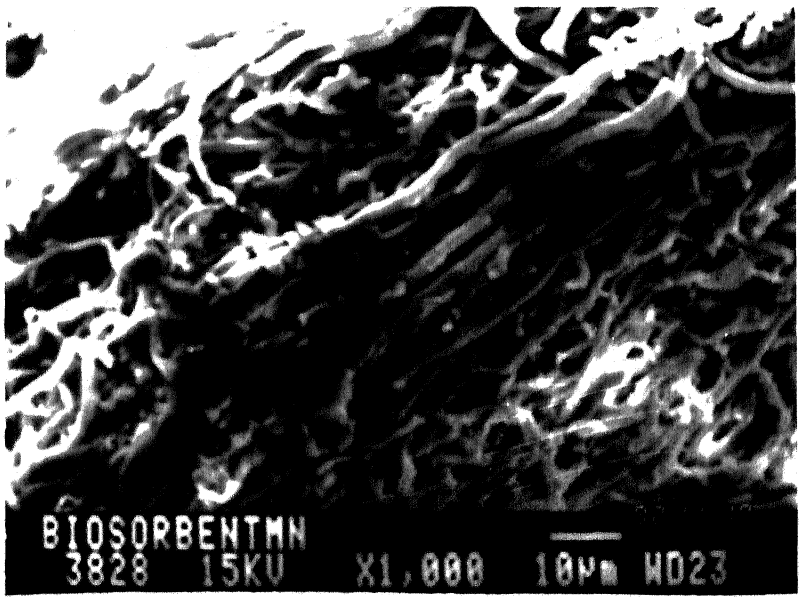


Fig. 5.26. Scanning Electron Micrograph of Biosorbent M_N.

5.3.3.3 Leachability Studies

The organic leachate from biosorbents is also of practical importance as it has been reported that there is leaching of organics from columns employing sorbents of biological origin (Freer and others, 1989). One of the methods suggested by these authors is the formaldehyde treatment. They, however, did not determine the yield of the biosorbent upon the pretreatment, and more importantly did not give comparison of leachability characteristics of the sorbent upon pretreatment *vis-a-vis* the untreated sorbent. The chemical oxygen demand (COD) of distilled water which was in contact with the biosorbents under specified experimental conditions could be taken as a gross measure of the strength of organic leachates. Results of the leachability characteristics of the biosorbents presented in Table 5.5 indicate a decrease in leaching of organics from 40 mg/L for M_1 to 16 mg/L for M_S measured as COD of leachate. It can also be observed that alkali treatment adversely affected the leaching characteristics. Observations of increasing decoloration of biosorbent upon contact with alkali has been reported by Kuyucak and Volesky (1989b).

5.3.3.4 pH of Biosorbent

If the biosorbent addition results in a drastic change in pH, it will interfere with metal uptake. The pH of the biosorbents as presented in Table 5.5 are all within narrow ranges around the pH of 7.0, indicating that the biosorbent addition in itself will result in only a small change of pH and consequently the impact on metal uptake will be minimal. This also implies that the treatment system need not be buffered to account for pH variation due to sorbent addition.

5.3.3.5 Stability Against Thermal Degradation

Effluents from electroplating industry, which are a major source of metallic pollutants, generally have a temperature of above 50^{o}C. Biomass may be degraded at elevated temperatures. It is therefore advantageous if the biosorbents are capable of maintaining its uptake properties at elevated temperatures. The experimental results with biosorbents heated to 180^{o}C and at an elevated pressure [1.089 kg/cm^{2} (guage pressure) and 120^{o}C] are presented in Table 5.5. Subjecting to elevated temperature have had no appreciable effects on the uptake performance. From an initial concentration of 1 mM, the change in uptake with reference to the sorbent not subjected to heat treatment were constant within a range of $\pm$ 5% . This is a highly desirable situation in that the biosorbent reactor can be attached on line to the effluent pipeline without the requirement of prior cooling as is necessary in the case of biosorbent reactors employing live microbes which are highly sensitive to temperature above 40 ^{o}C.

5.3.3.6 Physical Characteristics

Biosorbents traditionally have been developed along the lines of ion exchange resins and hence behavior in contact reactors are of significance. Applied either in Continuously Stirred Tank Reactor (CSTR) or in Packed Bed Reactors, the biosorbent is subjected to substantial force of attrition and it is important that the biosorbent have good physical strength. All biosorbent derivatives prepared from *G. lucidum* have excellent resistance to attrition with an average of more than 85% of the sorbents surviving the attrition test (Table 5.5).

One of the significant advantages of employing macrofungi as biosorbents is their natural size (average size diameter 150 mm and thickness 20 mm in the present case) and hence no immobilization is warranted. This ensures that the metal uptake capacity and kinetic properties observed in laboratory scale studies is maintained in a field scale reactor also. The adsorbents could be employed in any type of reactors, either a CSTR or a column as the biosorbents settles easily under gravity. The head loss features in packed bed reactors are discussed in following section.

The packing density of the biosorbent *G. lucidum* and its derivatives are presented in Table 5.5. The values vary from 180 g/L to 230 g/L. The biosorbents also have a specific gravity above 1.1.

5.3.3.7 Filterability and Head Loss Development in Packed Bed Reactors

The head loss developed in a biosorbent column at flow rates generally encountered in adsorption reactors is presented in Table 5.5. The maximum possible filtration rates under gravity is also presented. While the head loss is nominal in all cases at flow rates of upto 3.5 $m^3/m^2/h$, it may be noted that it is possible to achieve a flow rate of over ten times what is generally applied in packed bed adsorption reactors. An upper threshold of 3.5 $m^3/m^2/h$ has been suggested by the Environmental Protection Agency as a judicious balance between effective solid liquid contact and excessive head loss while employing carbon adsorption (EPA, 1973). The flow rates of effluents can therefore be enhanced substantially in the case of biosorbents.

From the tabulation of the physico-chemical characteristics of the biosorbent and its derivatives, it can be noticed that while there

is marginal improvement in selected parameters due to some treatment, no drastic enhancement of any of the desirable qualities has been achieved. Formaldehyde treatment as suggested by Freer and others (1989) did not substantially improve the leaching characteristics.

The above results, taken in conjunction with the metal uptake properties and the rate constants indicate that while chemical pretreatments could be given to selectively enhance the properties of the adsorbents, the biosorbent *G. lucidum* does not require any chemical treatment except simple washing before use for metal uptake. Considering the reduction in yield and also the cost involved in chemical pretreatment, the enhancement of metal uptake observed in the case of alkali treated *G. lucidum* also does not warrant further evaluation. It was therefore decided to conduct further investigations using biosorbents prepared from *G. lucidum* after pulverizing and sieving to a GM of 848 μm (600-1200 μm range). The terms biosorbent, M_1 and *G. lucidum* are hereafter used synonymously unless otherwise specified.

5.4 MECHANISM OF BIOSORPTION

As seen from the extensive survey of literature, even though the metal-microbe interaction has been investigated by researchers from various disciplines ranging from pure to applied sciences, a comprehensive understanding is yet to evolve. Mechanism studies can aid in manipulating the biosorbent organism at the genetic (Summers, 1985), propagation (Wong and Kwok, 1992) or application (Muzzareli and others, 1980) stage. An understanding of the nature of interaction between the biosorbent and the sorbate will also facilitate better process design and control. The knowledge regarding the mechanism of

biosorption combined with the selection of most appropriate reactor configuration based on it, is expected to result in an optimal treatment alternative for the hazardous metal bearing effluents.

The biosorbent employed in the present investigation being non-viable, the scope of mechanism study is limited to the elucidation of the following details.

1. The site of interaction
2. The type of adsorption process
3. The coordination environment of the metal within the biosorbent

To undertake a study on delineation of mechanism, which is of fundamental nature, is difficult in the case of biosorbent because of the presence of wide variety of surface groups and also the variation of binding mechanisms among the species. An attempt, however, is made to understand the mechanism of biosorption of copper (II) by *G. lucidum* using a combination of biochemical, microscopic and spectroscopic techniques.

5.4.1 Transmission Electron Microscope Studies

All species of phylum Fungi (Mycota) are composed not of cells as in the case of other organisms, but of cylindrical septate hyphae. An aggregate of hyphae is called mycelium. The walls of hyphae are semi rigid and most fungi may have more than one of the following type of hyphae.

1. Nonseptate hyphae: These have no septa or cross walls derived from the hyphal walls
2. Septate hyphae with uninucleate cells:These have septa throughout the hyphae. The hyphae are produced from ingrowth of hyphal wall which forms a peripheral ring within the hypha having a

central pore through which cytoplasm and nuclei can migrate from one compartment to another.

3. Septate with multi nuclear cells:These have more than one nucleus in each compartment.

It is to be understood that each compartment of septate hypha is not separated by a membrane, but by tradition each compartment is referred to as a cell. The terms like cell wall and hyphal wall are used synonymously.

The metal being taken up by a live cell can either be detained at the cell wall by extra cellular binding groups or can enter the cell by energy mediated (active) or facilitated transport processes. In the case of dead cells as used in this study, there is no possibility of an active transport. There could however be a facilitated transport of metals across the hyphal wall mediated by the concentration gradient.

Transmission electron microscope (TEM) studies have been employed to locate the biosorbed metal by many researchers (Tsezos and Volesky, 1982 a and b; Kuyucak and Volesky, 1989c; Mann, 1990). TEM pictures of the biosorbent *G. lucidum* before and after Cu(II) uptake were taken and the resulting photographs are presented in Figure 5.27 to 5.29. The TEM photographs of virgin hyphal wall presented in Figure 5.27 indicates that *G. lucidum* has an extremely thick hyphal wall with the cytoplasmic central pore. The extremely thick hyphal wall of *G. lucidum* is rather unusual but not unexpected as the fruiting bodies serve to function as spore forming and propagating agents with no other metabolic functions. The pictures (Figure 5.28 and 5.29) after metal uptake depicts an electron dense area of the entire hyphal wall indicating that the probable site for metal interactions is the hyphal

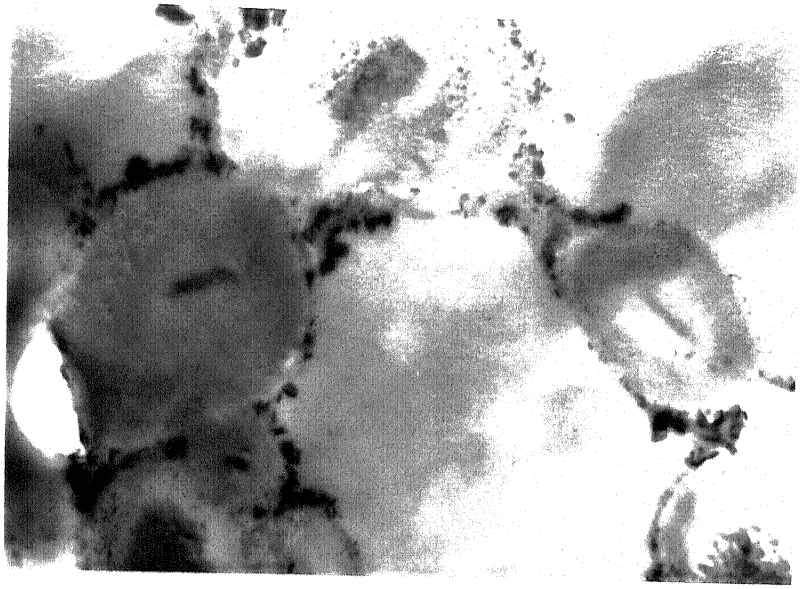


Fig. 5.27. Transmission Electron Micrograph of Biosorbent M_1 Before Cu(II) Uptake. (X 13000)

Fig. 5.28. Transmission Electron Micrograph of Biosorbent M_1 After Cu(II) Uptake. (X 13000)

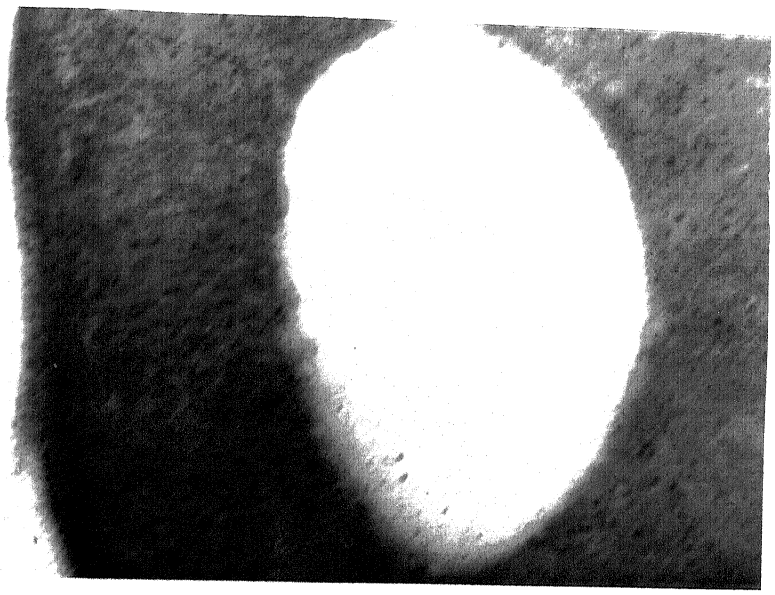


Fig. 5.29. Transmission Electron Micrograph of Biosorbent M_1 After Cu(II) Uptake. (X 39000)

wall. Similar results have also been observed for uranium biosorption onto non-viable *R. arrhizus* by Tsezos and Volesky (1982a). No metal has penetrated into the cytoplasmic layer as indicated by Figure 5.29. It can also be noticed that there is a uniform distribution of metal within the hyphal wall and no distinct layers are visible as observed by Tsezos and Volesky (1982a) for *R arrhizus*.

The hyphal walls are as rigid as the bacterial cell walls, though their chemical and structural characteristics are different (Brunnert, 1979). Most of the fungal cell walls are made up of polysaccharides which are complexed with proteins, lipids and some pigments. A schematic representation of a typical hyphal wall is presented in Figure 5.30. A typical composition of cell wall components of macrofungi as reported by Ledieu and Mendoza (1981) is presented in Table 5.6.

Table 5.6 Composition of Typical Hyphal Wall*

Component	% of Total
Hexosamines	36
Neutral Carbohydrates	38
Proteins	10.2
Lipids	
Readily Extractable	4.7
Bound	5.2
Nuclic Acids	0.3
Chitin/Chitosan	5.6

* Lidieu and Mendoza, 1981

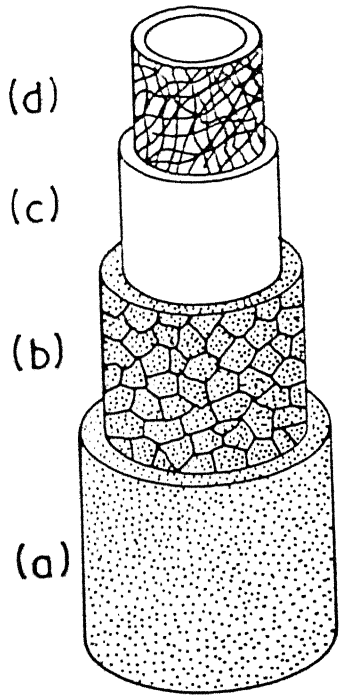


Fig. 5.30. Schematic Structure of the Cell Wall of A Typical Fungus. (Ref. Brunnert, 1979)

(a) Outer Mixed α and β Glucons.
(b) Glycoproteins and Glucons Merging into Proteinaceous Material.
(c) Primary Proteinaceous Material.
(d) Inner Chitinous Region.

The fungal cell walls presents a multi laminate, microfibriliar structure with an outermost layer of lipids followed by a layer of glucans which merge into an interior layer of structural proteins. The main structural element is a polysaccharide layer which is conferred by the parallel arrangement of chitin chains, cellulose chains or in other cases non-chitinuous polysaccharides. There is a continuous transition between these layers. In addition polyphosphates and inorganic ions are also found in fungal cell wall.

5.4.2 Identification of Metal Binding Component in the Hyphal Wall

Metal adsorbed by the hyphal wall could be accumulated onto any one or all of the layers described above. The identification of the specific component of the hyphal wall which accumulates the metal is of immense practical value, because it offers an opportunity to elute all the unwanted components and/or selectively concentrate desirable components. Further, such a study would facilitate to increase the metal binding cellular components by changing the growth environment during its propagation (Treen-Sears and others, 1986).

Identification of the component(s) participating in metal binding was attempted by a combination of biochemical and spectroscopic techniques. Since the cytoplasm is only a fraction of the total cell mass and also as it is evident from TEM studies that it did not accumulate metal, no further attention was given to it. Thus the further study pertained to cell wall.

5.4.2.1 Selective Elution of Cell Wall Components

Elution of the lipid content of the cell wall was carried out using a mixture of methanol and chloroform. The uptake

capacity (apparent and corrected for weight loss) presented in Table 5.3 indicate that lipids do not appear to take part in metal uptake by the biosorbent *G. lucidum.*

The elution of proteinaceous matter achieved by cold alkali treatment (sorbent M_3) resulted in a drop of metal uptake capacity to the tune of only 3.4 %. This is indicative of the limited role played by proteins in metal uptake by *G. lucidum.*

Treating the biosorbent M_3 with strong alkali, however, resulted in a reduction of metal uptake by 18.4 %. Treatment with such strong caustic solution can result in the removal of most of the cell wall components except the extremely recalcitrant structural proteins and polysaccharides. Therefore it can be speculated that most of the metal uptake (81.6 %) is achieved by the innermost layer of structural polysaccharides.

The quantitative measure of metal uptake cannot however be taken as a comprehensive evidence regarding the role of the metal binding sites. The uptake capacity obtained being a gross parameter, it probably represents the total impact of the chemical treatments. The following changes may result to biosorbents upon chemical treatment.

1. Elimination of cellular components as envisaged and their individual impact on metal uptake
2. Unmasking of cellular groups, hitherto not participating in metal uptake

Further differentiation of these two possibilities requires detailed instrumental analysis which is presented in the succeeding sections

5.4.2.2 Infrared Spectroscopic Studies

Infrared spectra can yield valuable information regarding the chemical groups possessed by the biosorbents. This technique has been extensively used in biosorption studies by earlier workers (Muzzareli and others, 1980, Tsezos and volesky, 1982a and b; Kuyucak and Volesky, 1989c) for identification of functional groups on the biosorbent.

The IR spectra of the adsorbents M_1, M_2, M_3 and M_4 are presented in Figure 5.31. The most striking aspect that can be inferred from the spectra is that no new group have been unmasked as a result of various chemical treatments imparted to the biosorbent M_1. The prominent groups signals are around wave numbers 3390, 2925, 1650 and 1050 cm^{-1}. The signals at 3390 cm^{-1} are most probably due to N-H stretching vibrations whose bending components are also present at 1650 cm^{-1} thus providing evidence for the presence of amino groups. The consistent presence of amino groups in all derivatives could be from proteins in derivatives M_1 and M_2, and from complex amino-polysaccharides in M_3 and M_4 which are residues after protein elution. The bands at 2925 cm^{-1} are indicative of O-H stretching in a carboxylic acid which also forms COO^- stretching in 1250- 1350 cm^{-1} region. The presence of carboxylic groups in algae *Chlorella vulgaris* and their active role in biosorption has also been reported by Majidi and others (1990)

5.4.3 Metal Coordination on the Hyphal Wall

5.4.3.1 Electron Paramagnetic Resonance Spectroscopy

Identification of metal coordinating environment in the biosorbent is an essential step in the delineation of mechanism of biosorption. If no new groups have emerged upon

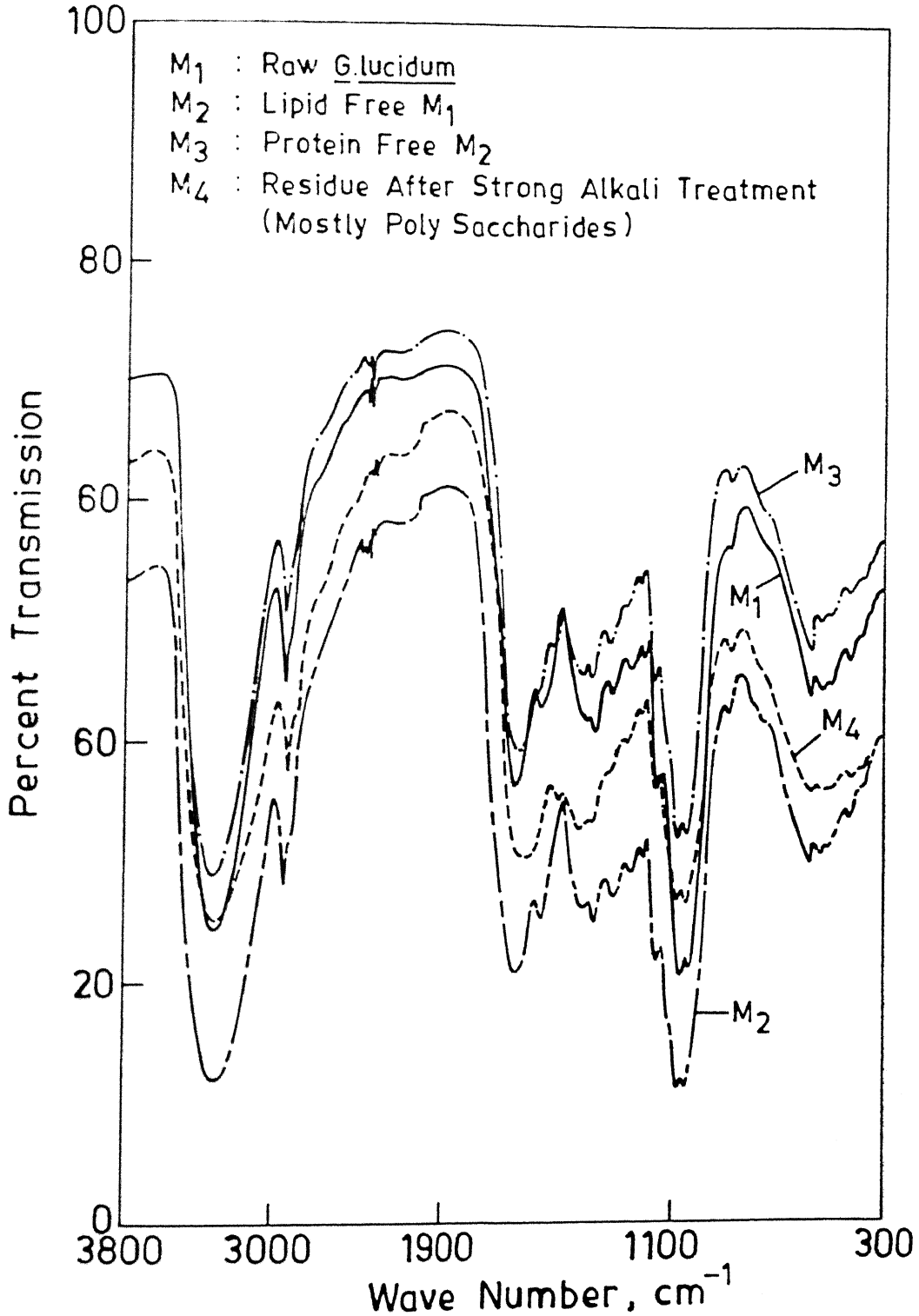


Fig. 5.31. Infrared Spectra for Various Biosorbents.

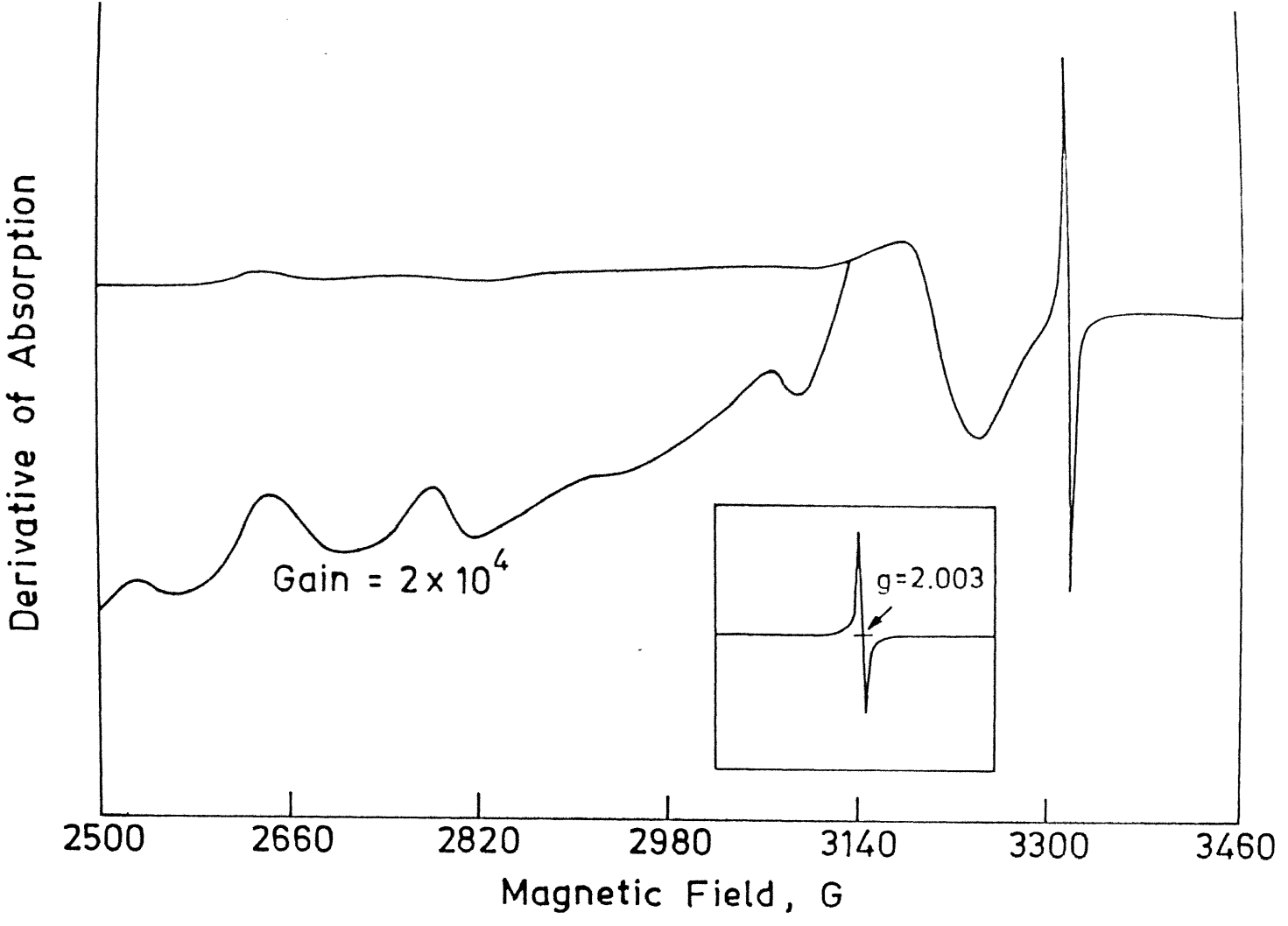


Fig. 5.32. EPR Spectrum for Biosorbent M_1 Loaded with Copper (II). Inset EPR for Biosorbent M_1.

Instrument Setting :

Time Const. 0.032 Mod. Amp. 10.0 G Gain 10×10^3 Power 10 mW

Scan Time 16 min Mod. Freq. 100 KHz Temp. 24 °C Freq. 9.37 GHz

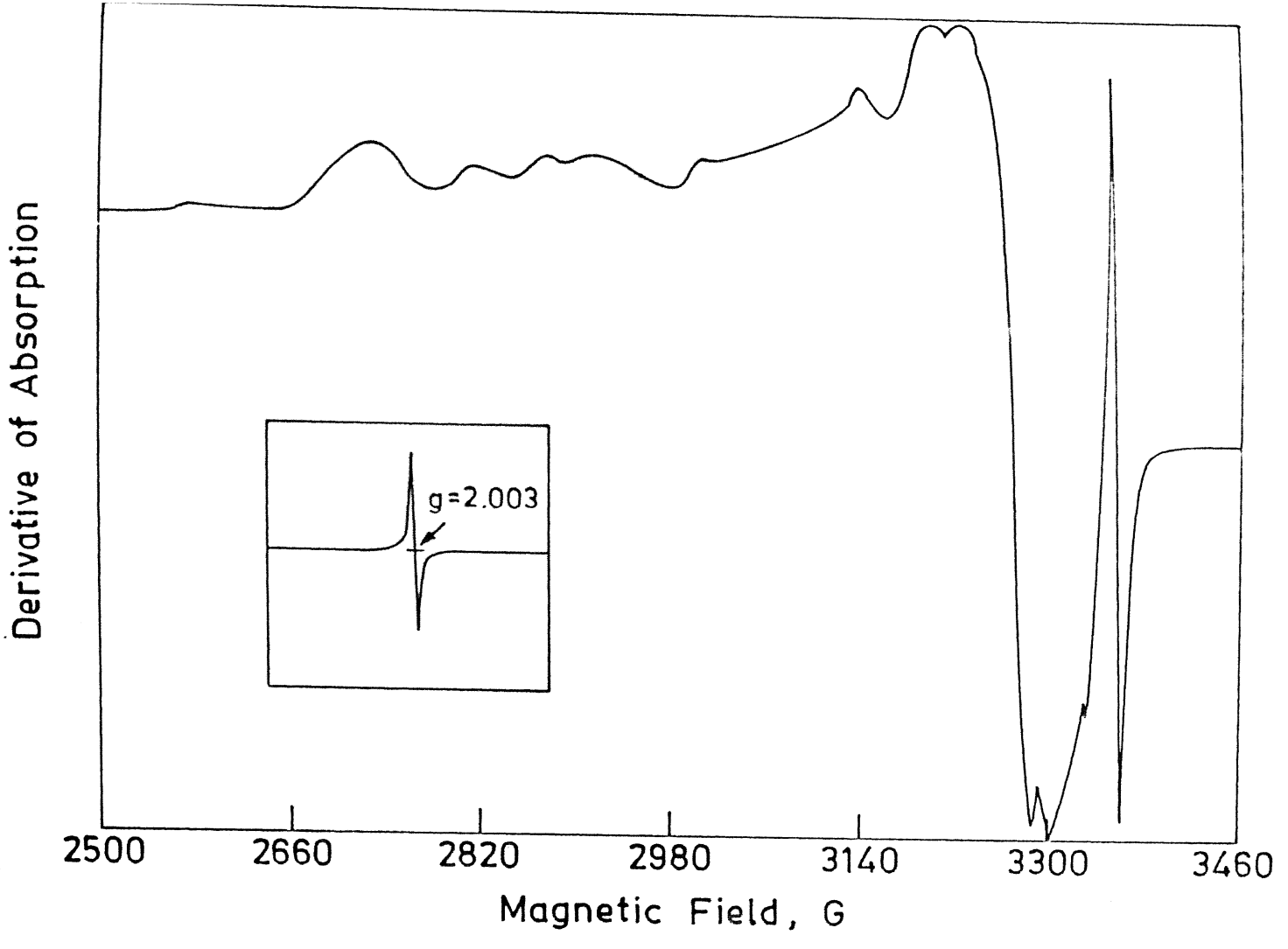


Fig. 5.33. EPR Spectrum for Biosorbent M_2 Loaded with Copper (II). Inset EPR for Biosorbent M_2.

Instrument Setting :

Time Const. 0.032 Mod. Amp. 2.0 G Gain 6.3×10^3 Power 2 mW

Scan Time 8 min Mod. Freq. 100 KHz Temp. 24 °C Freq. 9.4 GHz

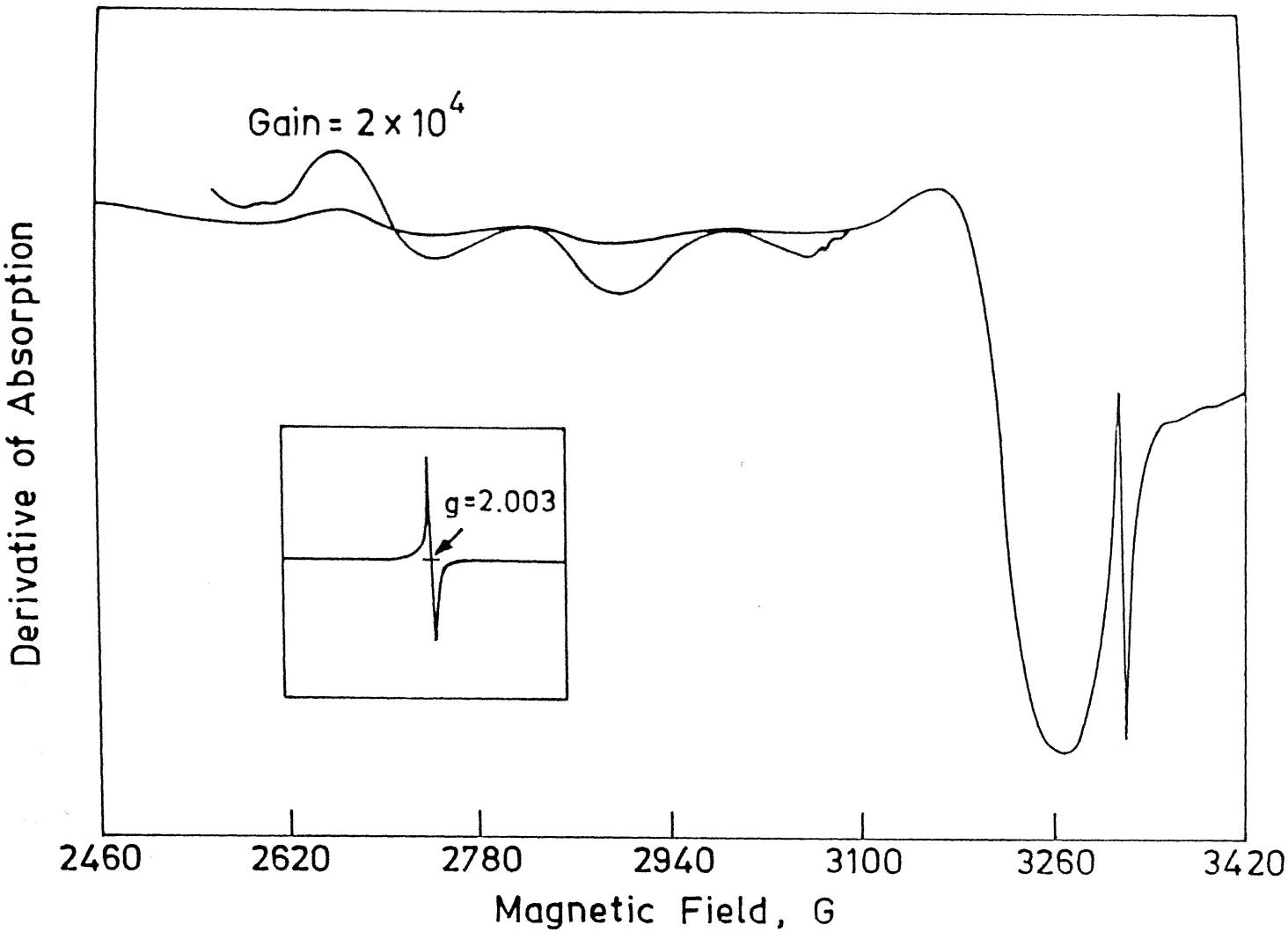


Fig. 5.34. EPR Spectrum for Biosorbent M_3 Loaded with Copper (II). Inset EPR for Biosorbent M_3.

Instrument Setting :
Time Const. 0.032 Mod. Amp 5.0 G Gain 10×10^3 Power 5 mW

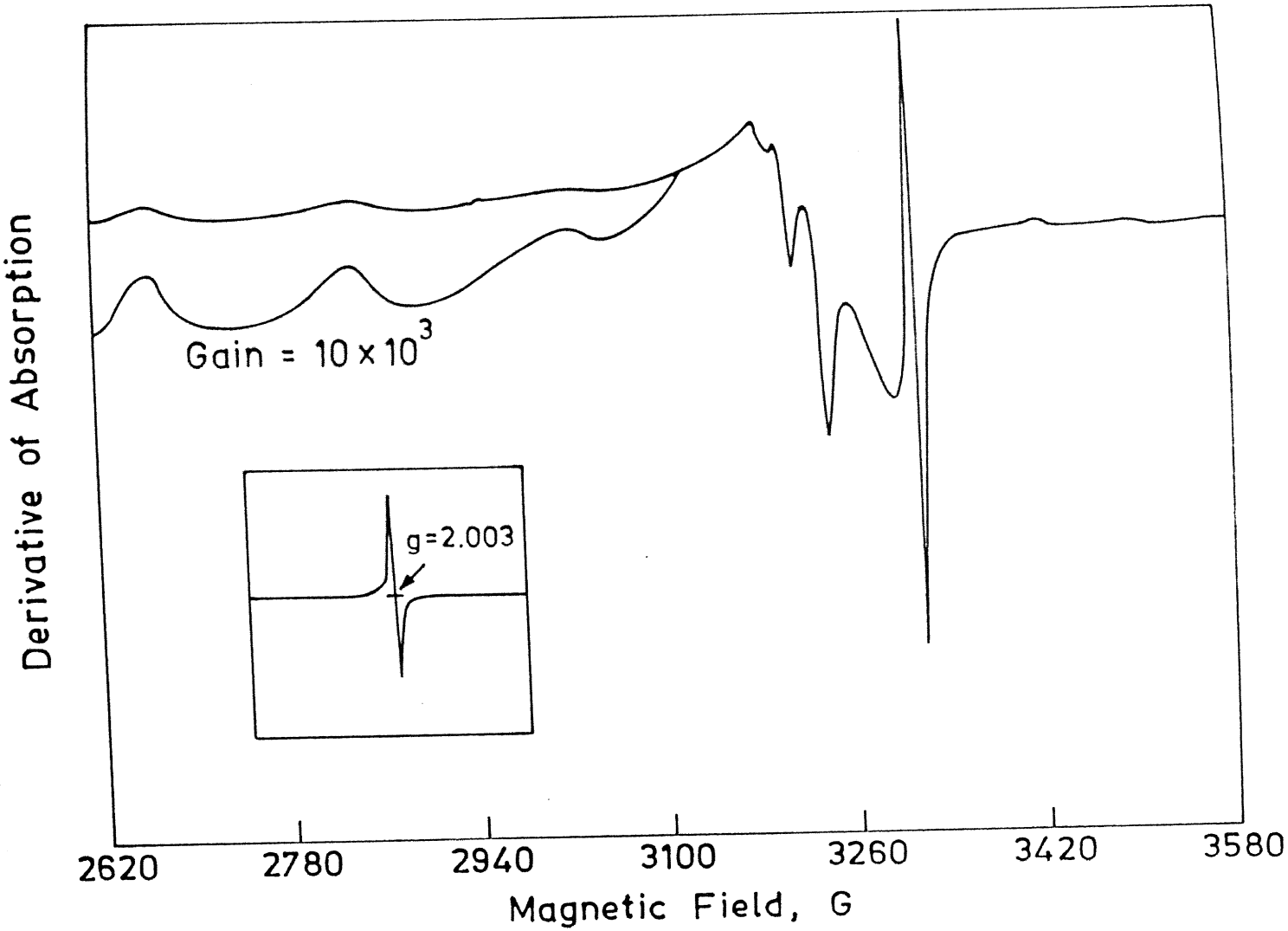


Fig. 5.35. EPR Spectrum for Biosorbent M_4 Loaded with Copper (II). Inset EPR for Biosorbent M_4.

Instrument Setting :

susceptible to the chemical treatments employed. Viewed in conjunction with the result of biochemical methods of elution, the matrix in which the free radical is trapped appears to be the structural polysaccharide level. While the hyperfine splittings of copper (II) signal in the case of M_1, M_2 and M_3 after sorption of Cu(II) give an indication about the geometry of the coordination environment, the spectrum for M_4+ Cu(II) yielded some very useful information. This spectrum exhibits the super hyperfine splittings in the $g_\perp$ region also, the resolution of the finer splittings perhaps is a result of the elimination of interfering groups besides purification of the material from a spectral point of view. There is a fourfold splitting in the $g_\perp$ signal which is immediately followed by the free radical signal appearing consistently at $g = 2.0038$.

EPR spectra similar to Figure 5.35 has been observed by Windle and others (1963) while studying the paramagnetic properties of copper centers in conalbumin. The spectrum as reported by the authors is presented in Figure 5.36 (a), and its similarity with M_4+ Cu spectrum can easily be noted. Five finer splittings on the $g_\perp$ region were attributed to the nitrogen interaction from two coordinating atoms. Coordination with two nitrogen will result in 2x2+1 (5) splittings, with the intensity ratios of 1:2:3:2:1. However, the authors were not able to obtain any quantitative analysis of the superhyperfine splitting which fit the above pattern. The overall coordination environment was suggested by Windle and others (1963) as one consisting of two nitrogen and two oxygen and is presented in Figure 5.36 (b). The EPR spectrum corresponding to a coordination environment having two nitrogen and two oxygen atoms were experimentally simulated by Wiersema

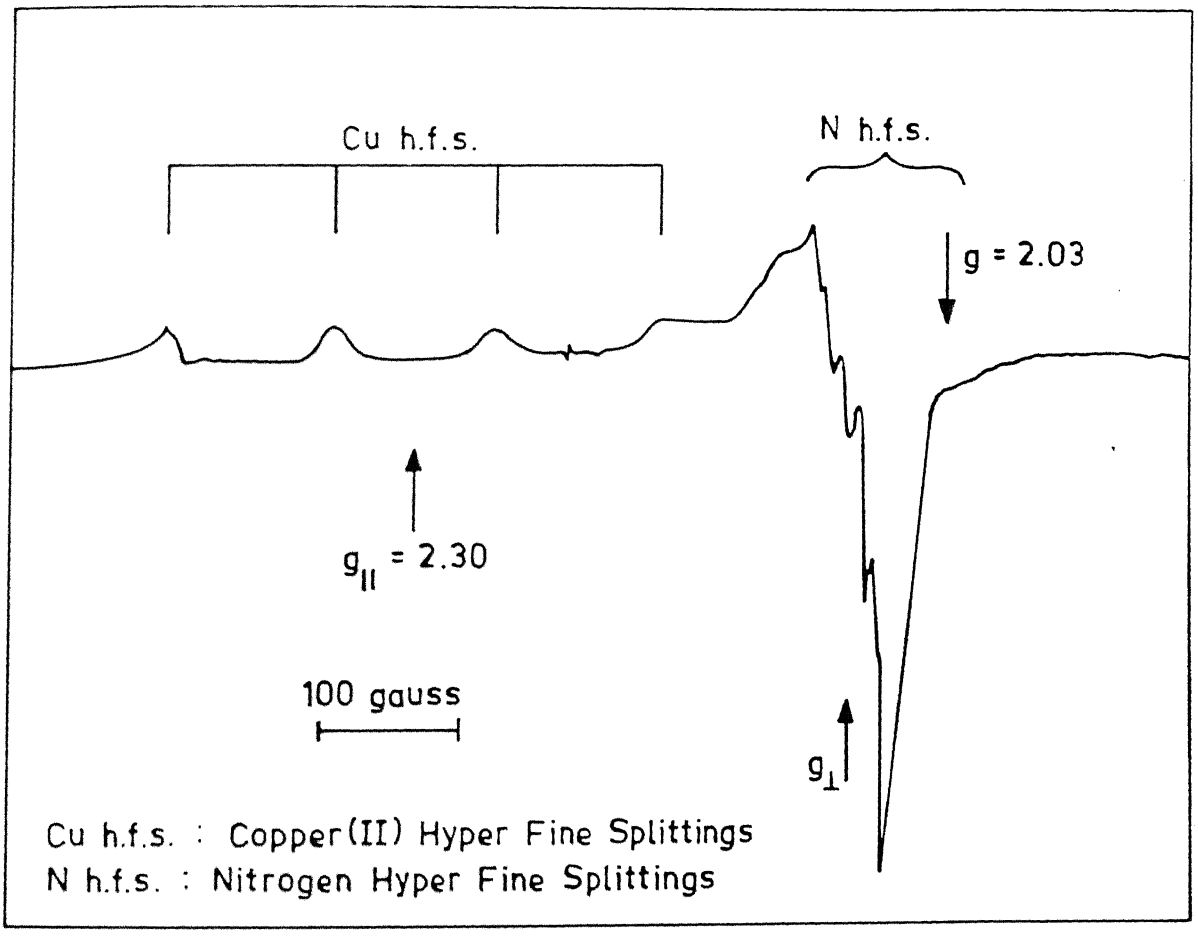


Fig. 5.36(a). EPR Spectrum from Solution of Copper Conalbumin. (Ref. Windle and Others, 1963)

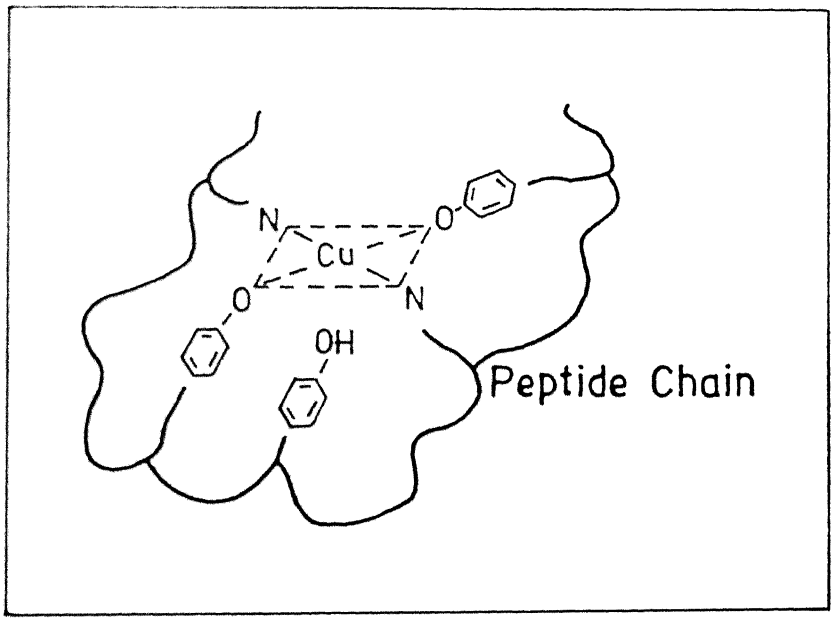


Fig. 5.36(b). Suggested Environment of Cu(II) Coordination in Conalbumin. (Ref. Windle and Others, 1963)

and Windle (1964) (Figure 5.37). Using copper complexed with salicylamide (structural formula shown in Figure 5.37), a five fold splitting of the copper signal in the $g_{\perp}$ region was obtained confirming the above hypothesis.

The EPR spectrum of M_4+ Cu(II), is similar to that obtained by Windle and others (1963) (Figure 5.36 (a)), though only four splittings are visible, the fifth one most probably has merged with the free radical signal which also falls in the same region. In light of the similarity of the EPR signals obtained in the present study and other investigations, it seems to be appropriate to assume that the copper is coordinated to two oxygen and two nitrogen atoms.

One interesting approach to the analysis of EPR spectra for Cu(II) adsorption has been suggested by Motschi (1985). Since copper is one of the strongest complex formers among metals and the unpaired electron in the $d_{x^2-y^2}$ has a maximum spin density directed towards the ligand places which renders the EPR parameters (g and A) sensitive to ligand variations, they proposed a correlation of $g_{\parallel}$ with the overall stability constant (log K_{tot}). The plot as suggested by the author is reproduced in Figure 5.38 incorporating the $g_{\parallel}$ values of biosorbent M_1. The biosorbent, having high stability constant as seen from Figure 5.38 indicates a strong complexation between copper (II) and the biosorbent M_1.

5.4.3.2 Energy Dispersion Analysis by X-Ray of Sorbent

EDAX technique can beneficially be employed to understand the elemental composition of the adsorbent. In the present study X-ray dispersion analysis of the biosorbent *G. lucidum* was conducted before and after adsorption of copper on to it. The

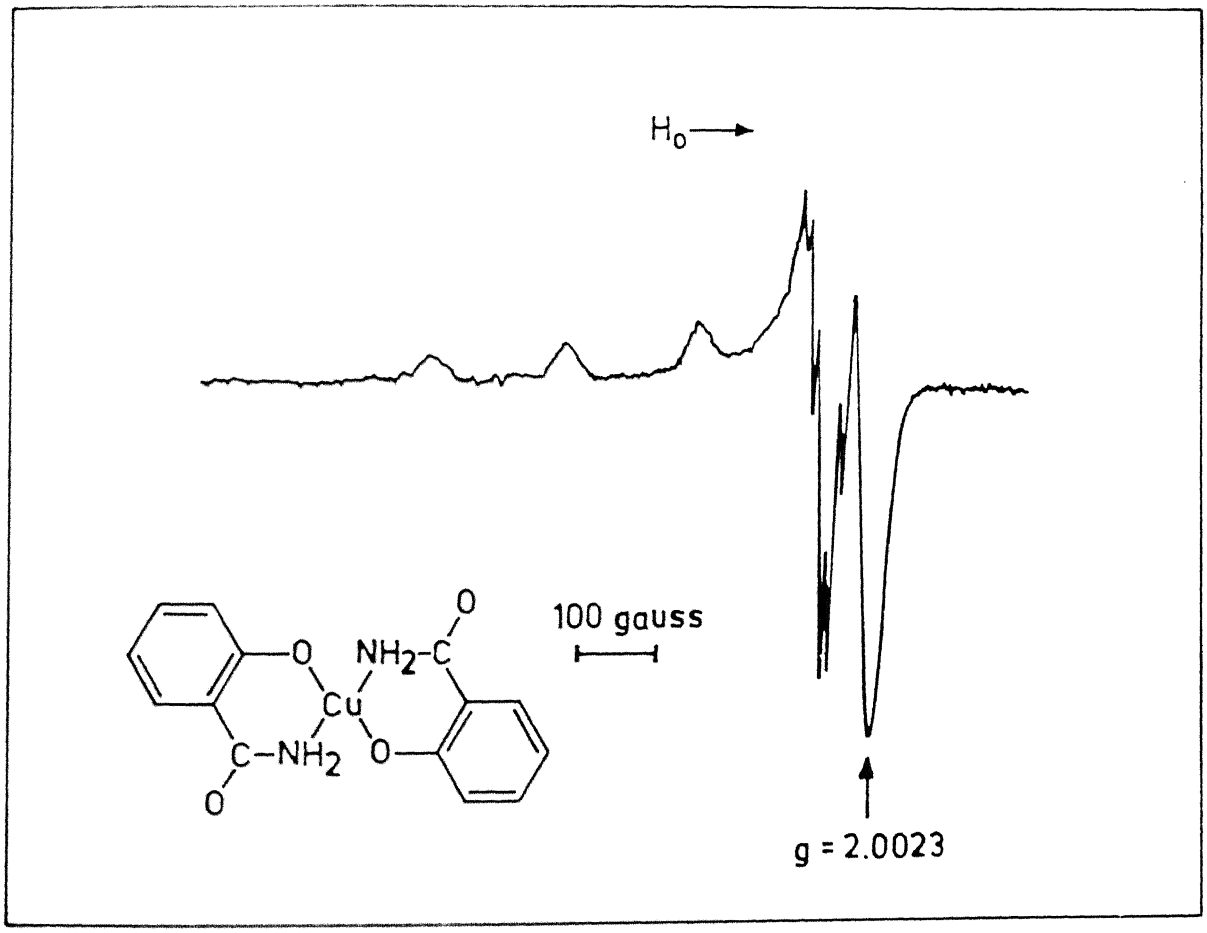


Fig. 5.37. Low Temperature Spectrum of Copper Salicylamide. (Ref. Wiersema and Windle, 1964)

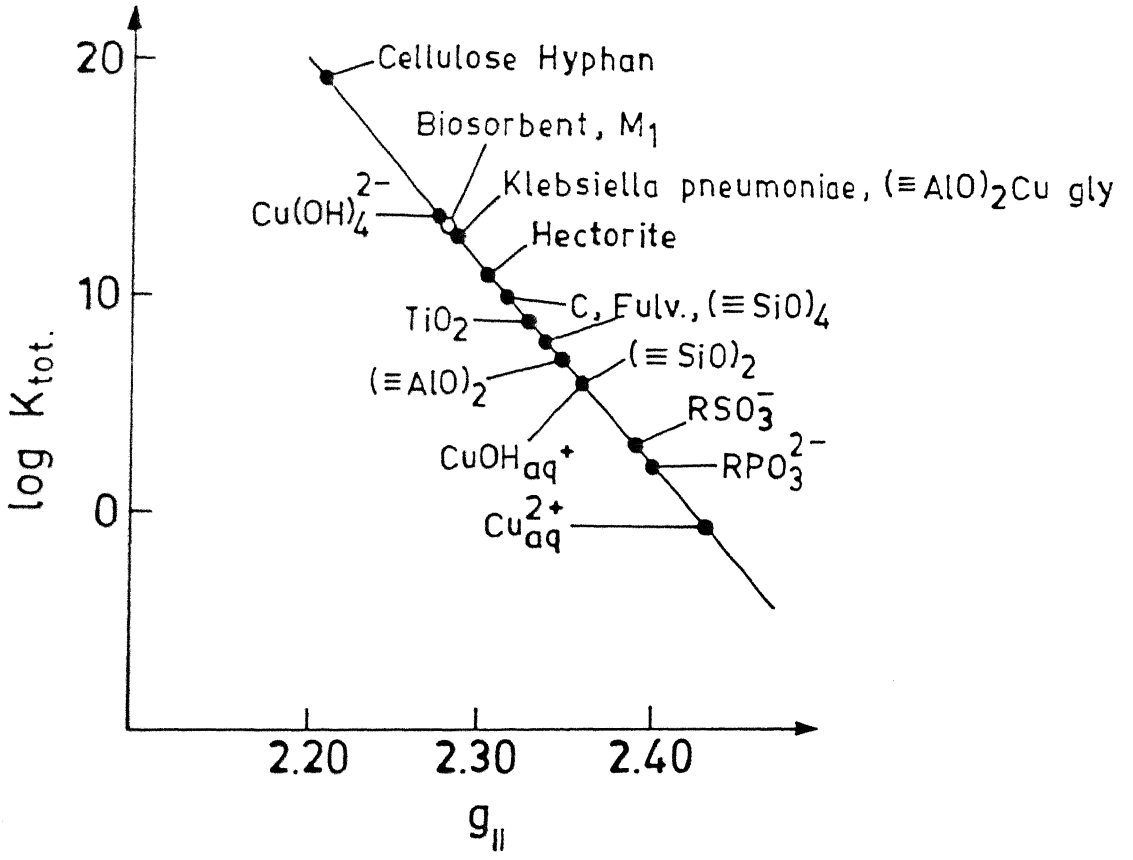


Fig. 5.38. Relation Between Splitting Constant (g_{II}) and Stability Constant (log K_{tot}) of Cu(II) Complexes. (Ref. Motchi, 1985)

resultant spectra are presented in Figure 5.39 and 5.40. The EDAX spectrum of biosorbent M_1 before metal uptake exhibits a distinctive calcium peak at 3.75 KV, indicating the presence of substantial amount of calcium in the adsorbent. After copper (II) uptake, however, the calcium peak is no longer predominant and copper signal is now distinctive (at 8.0 KeV) (Figure 5.40). This indicates the possibility of copper replacing the calcium from the cellular material.

Apart from the basic building blocks (C,H,N,S and P) calcium is the most abundant constituent of biological systems. Calcium is found principally in extracellular locations, its main function being the stabilization of structural components of cells, eg., extracellular proteins, cell membranes and cell walls (Williams, 1981). It is therefore likely that cellular components responsible for biosorption, had calcium in their molecular structure. The amount of calcium present in the biological material was determined by eluting it with mineral acid. It was found to be 12 mg of calcium/g of adsorbent, (0.3 mmol of Ca/g) whereas the amount of copper being adsorbed is 0.383 mmol/g suggesting a near stoichiometric exchange of copper for calcium ions. Further, calcium was not detected in identical sorption solution without copper when the adsorbent was agitated in the control experiments. This suggests that calcium release is the result of copper uptake by *Ganoderma lucidum*. The presence of calcium and magnesium in virgin cells of *Ascophyllum nodosum* has been reported by Kuyucak and Volesky (1989c). It was also observed by the above authors that the amount of Ca^{2+} and Mg^{2+} released increased with the increase in cobalt uptake by the algae.

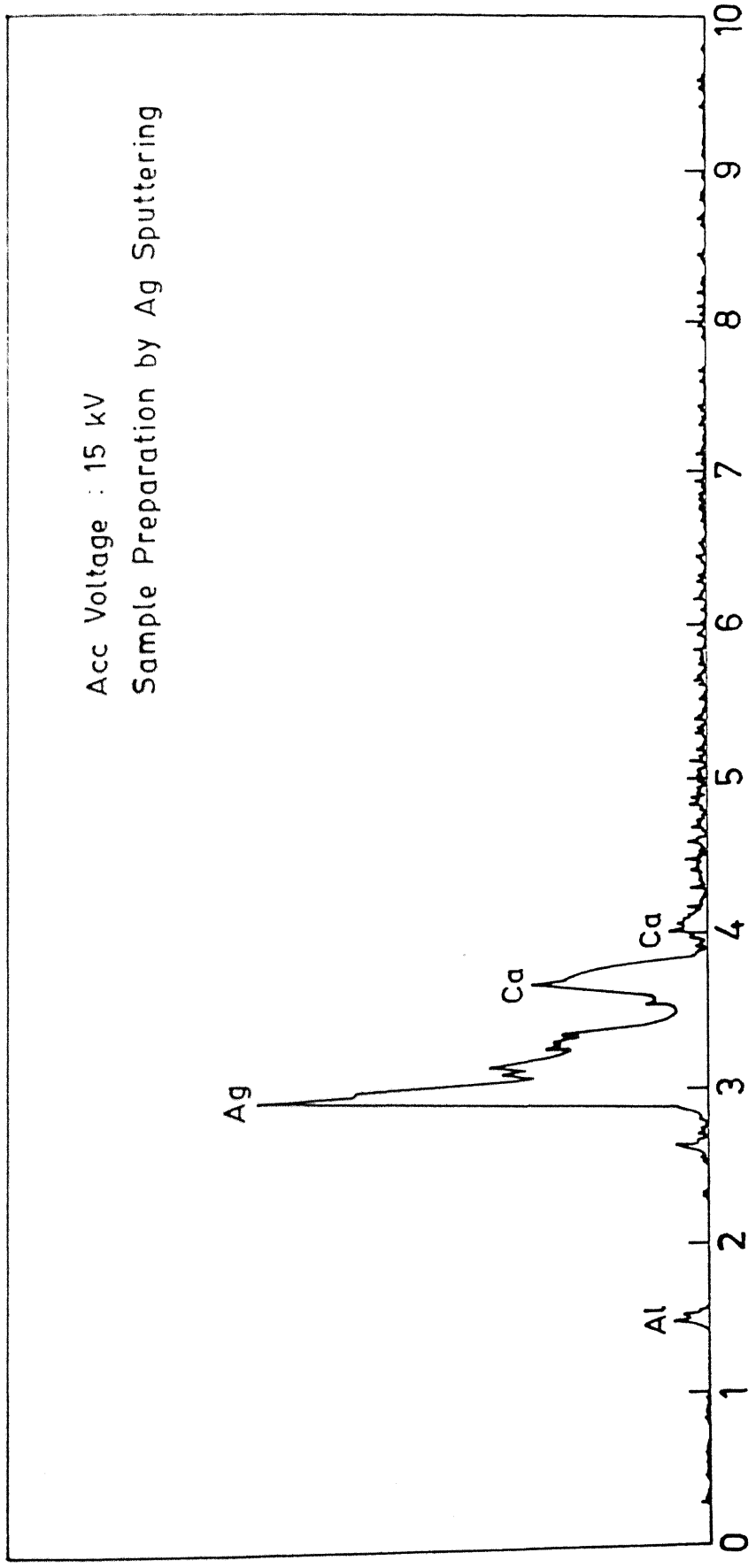


Fig. 5.39. EDAX Spectrum of Biosorbent M_1.

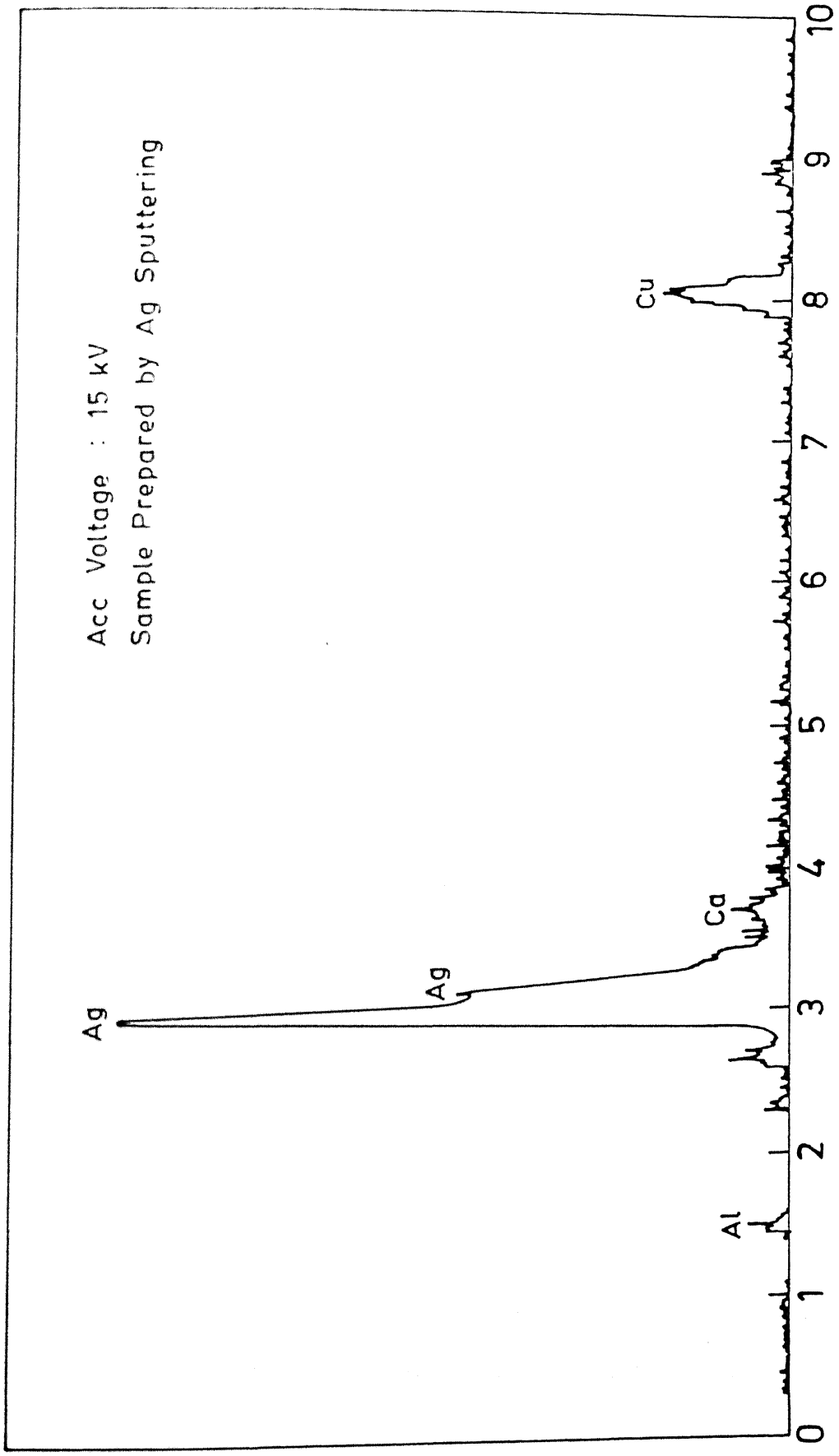


Fig. 5.40. EDAX Spectrum of Biosorbent M_1 After Cu(II) Uptake.

5.4.3.3 Variation of pH During Biosorption:

The pH of the unbuffered reaction mixture (1 mM Cu(II) in triple distilled water) upon biosorption was monitored continuously to determine whether H^+ ions were being released into the aqueous medium. The kinetics of pH change is presented in Figure 5.41. A drop in pH from an initial value of 5.8 to 4.03 in first fifteen minutes was observed whereafter the pH was almost constant. The change in pH in the triple distilled water (pH 7.0) into which raw biosorbent was added was only marginal. The decrease in pH appears to be due to the release of H^+ ions from the biosorbent into the aqueous phase upon sorption. Groups like carboxylic and sulfhydral readily exchange protons for other ions in biological systems like *G. lucidum.* Similar observations has also been reported by Fletcher and Beckett (1987a), and the authors utilised this property to elute copper (II) back into the solution by reducing the pH of the aqueous medium. In the present study a quantitative evaluation of H^+ efflux indicated that 0.048 mM of H^+ ions were released per every mM of copper taken up, which is equivalent to about 10% of the copper removal by the biosorbent.

The total amount of calcium and hydrogen ions available for exchange with copper can be added up and this come to 0.348 mmol. However the total metal uptake is of the order of 0.383 mmol/g. The efflux of no other ion was observed and thus it appears that some mechanism other than ion exchange is also active in biosorption of copper by *G. lucidum.* Ion exchange is a very fast reaction and the non-cessation of metal uptake even after fifteen minutes also support the hypothesis that mechanisms other than ion exchange may also be

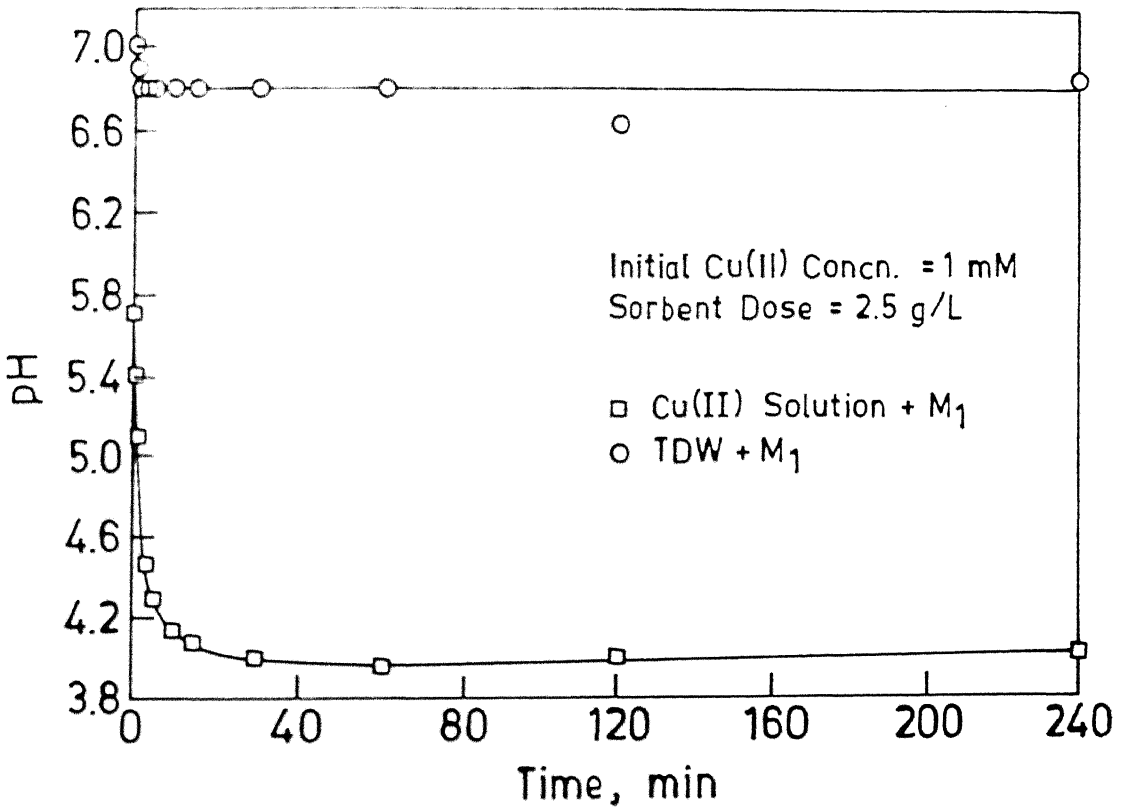


Fig. 5.41. Effect of Incorporation of Cu(II) in the Sorption Solution on Aqueous Phase pH.

operative in the biosorption of copper (II) by *G. lucidum*. The extent of this type of mechanism is however marginal.

5.4.3.4 Reversible Kinetics Theory of Ion-exchange

The sorption of metals from liquid phase to solid phase may be considered as a reversible reaction with an equilibrium being established between the two phases. Helferich (1962) suggested a simple first order reversible kinetics model to establish the rates of reaction. The model was originally proposed for ion exchange reactions. In view of the confidence gained about the mechanism of biosorption and its similarity with ion exchange, it was considered appropriate to fit this model to the kinetic data of copper (II) uptake by *G. lucidum*. The simple reversible reaction of adsorption of an adsorbate from solution (A) to sorbent (B) can be expressed as

$$A \underset{k_2}{\overset{k_1}{\rightleftharpoons}} B \qquad 5.11$$

where A and B are the concentration of the adsorbate in solution and sorbent phase. When the first order reversible kinetics holds true (as in the case of ion exchange reaction for example), the rate equation for the reaction is expressed as

$$-\frac{dC_A}{dt} = \frac{dC_B}{dt} = C_{Ao}(dX_A/dt) = k_1 C_A - k_2 C_B \qquad 5.12$$

$$= k_1 (C_{Ao} - C_{Ao} X_A) - k_2 (C_{Bo} + C_{Ao} X_A) \qquad 5.13$$

where C_A and C_B are the concentrations of the metal at any time in the solution and on the sorbent, C_{Ao} and C_{Bo} are the initial concentrations of the metal in solution and on sorbent respectively. X_A is the fractional binding of the metal to sorbent and k_1 and k_2 are the first order rate constants.

At equilibrium

$$-dC_A/dt = dC_B/dt = 0 \qquad 5.14$$

and hence

$$X_{Ae} = \frac{K_c - (C_{Bo}/C_{Ao})}{K_c + 1} \qquad 5.15$$

where X_{Ae} is the fractional conversion of adsorbate at equilibrium and K_c is the equilibrium constant defined as

$$K_c = \frac{C_{Be}}{C_{Ae}} = \frac{C_{Bo} + C_{Ao}X_{Ae}}{C_{Ao} - C_{Ao}X_{Ae}} = \frac{k_1}{k_2} \qquad 5.16$$

where C_{Ae} and C_{Be} are the equilibrium concentrations of metal in solution and on surface respectively. The rate equation in terms of equilibrium conversion can be obtained from equation

$$dX_A/dt = (k_1 + k_2)(X_{Ae} - X_A) \qquad 5.17$$

integrating and substituting for k_2 from equation 5.16, the following relation is obtained

$$-\ln [1 - (X_A/X_{Ae})] = k_1 [1 + (1/K_c)] t \qquad 5.18$$

substituting $X_A/X_{Ae} = U(t)$ and $k_1 [1 + (1/K_c)] = k'$

equation 5.18 transforms to

$$\ln [1- U(t)] = -k' t \qquad 5.19$$

where $k' = k_1 + k_2$, defined as the overall rate constant

and $U(t) = (C_{Ao} - C_A)/(C_{Ao} - C_{Ae})$, defined as the fractional attainment of equilibrium

If ln [1-U(t)] is plotted against time, it should result in a straight line, in case biosorption obeys the underlying assumption of reversible kinetics. The adsorption kinetics of uptake of Cu(II) by *G. lucidum* linearised according to the above model is presented in Figure 5.42. Instead of a single linear profile, the kinetics exhibits an initial linear portion which deviates after 15 minutes. The behavior of the curve indicates that the reversible kinetics is followed during the initial period. This trend is expected in view of the observations of pH change as well as calcium exchange, all of which pointed to the fact that bulk of the uptake is by ion exchange.

5.5 RATE LIMITING PROCESS IN BIOSORPTION

Biosorption is a time dependant process and the rate of uptake is a function of the different steps involved. The rate equation of adsorbate transport in biosorbent can be developed similar to the equations proposed by Boyd and others (1947) for ion exchange adsorption process.

5.5.1 Diffusion Through Liquid Film

An adsorbent, whether an ion exchange resin or biosorbent, consists of relatively large, porous particles. The exchanging groups responsible for the adsorptive capacity of these materials are dispersed uniformly throughout the exposed surfaces. In

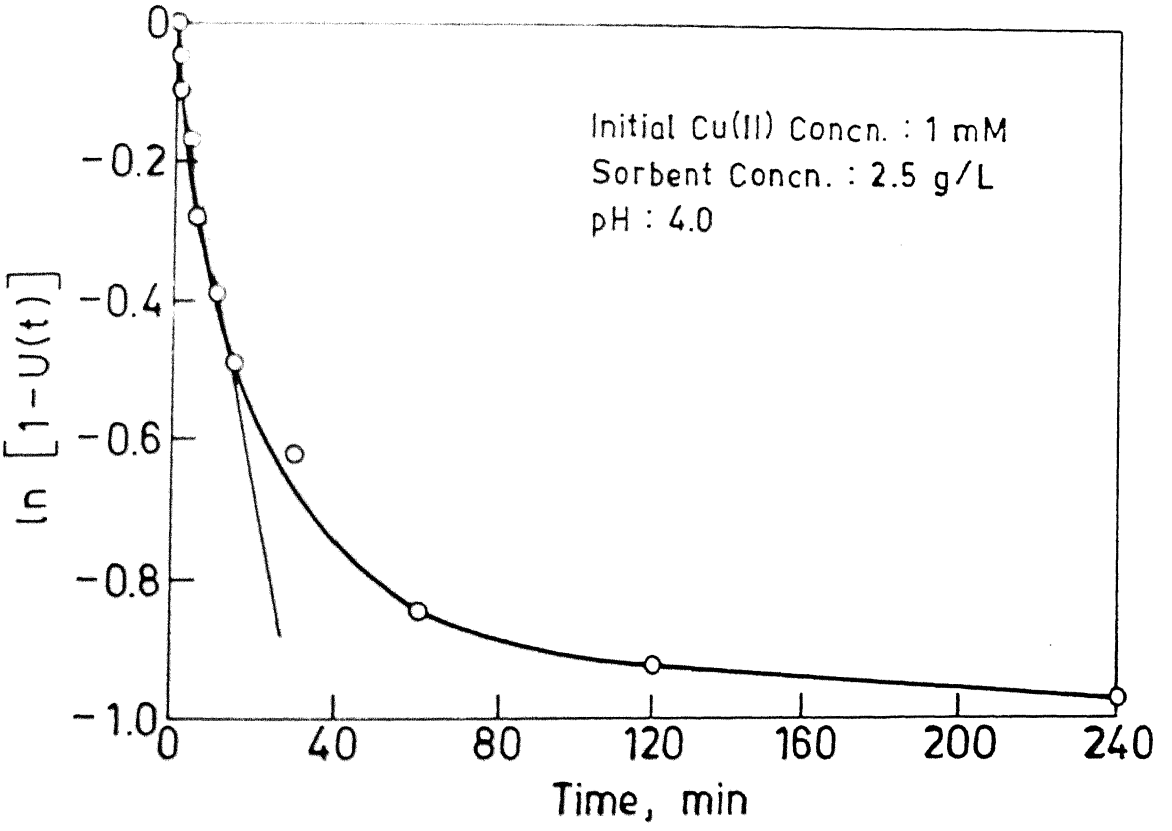


Fig. 5.42. First Order Reversible Kinetic Fit of Cu(II) Adsorption on G.lucidum.

terms of exchange reaction

$$A^{2+} + BR \rightleftharpoons AR + B^{2+} \qquad 5.20$$

where A^{2+} and B^{2+} are the exchanging cations, and R refers to the non-diffusible anionic portion of the adsorbent, the overall transport of mass, apart from that effected by moving liquid, may be divided into five steps:

1. Diffusion of A^{2+} through the solution upto the adsorbent particle
2. Diffusion of A^{2+} through the adsorbent particle
3. Chemical exchange between $A^{2+}$and BR at the exchanging position in the interior of the particles
4. Diffusion of the displaced cation B^{2+} out of the interior of the exchanger (reverse of step 2)
5. Diffusion of the displaced cation B^{2+} through the solution away from the adsorbent particle (reverse of step 1).

The kinetics of the exchange will be governed either by a diffusion or by a mass transfer mechanism, therefore, depending on which of the above steps is the slowest. An understanding of the rate limiting step in adsorption will greatly aid the selection of reactor configurations and also the time of contact to be allowed between the sorbent and sorbate. Generally, limitation by step 1 and 5 is eliminated by vigorous mixing in a batch reactor and allowing higher flow rates in a packed bed reactor. However, if the uptake is very rapid, it may not be possible to transport ions to the boundary at a rate sufficient to realize this desired condition. Then a liquid film in which a concentration gradient persists may be imagined to envelop the particle.

Consider a spherical adsorbent particle of radius r_o, surrounded by a sphere of aqueous solution of radius r_o', in which a concentration gradient exists, (Figure 5.43). Let $\Delta r_o = r_o' - r_o$, be taken as the thickness of the film. Let C^l, the concentration in the bulk of the solution at any time and constant for $r \geq r_o'$; and C^f, the concentration in the film taken to vary linearly from $C^f_{r=r_o'} = C^l$ to $C^f_{r=r_o} = C^{l*}$, the concentration in the solution adjacent to and hence in equilibrium with the solid. The concentration in the solid, C^s, is assumed constant in the region $0 \geq r \geq r_o$. Further denote the final equilibrium concentration in the solid and liquid by C^s_e and C^l_e respectively. The distribution coefficient, $\varkappa$, which is assumed independent of concentration is defined by $C^s_e = \varkappa C^l_e$ (This assumptions is valid only in dilute solutions, which is applicable in the present case). In order for $\varkappa$ to be constant within experimental error, however, it is sufficient if the adsorbate be a micro component of the system.

Equilibrium at the surface of the adsorbing particle is assumed for all times of contact so that for $r = r_o$

$$C^f_{r=r_o} = C^s/\varkappa = \frac{Q}{\frac{4}{3}\pi r_o^3 \varkappa} \qquad 5.21$$

since the total amount of adsorbate per particle Q is given by $(4/3)\pi r_o^3 C^s$.

If diffusion through the film is rate controlling, the rate of permeation, P (ie., quantity transferred /unit time/unit area of unit thickness under a standard concentration difference) is

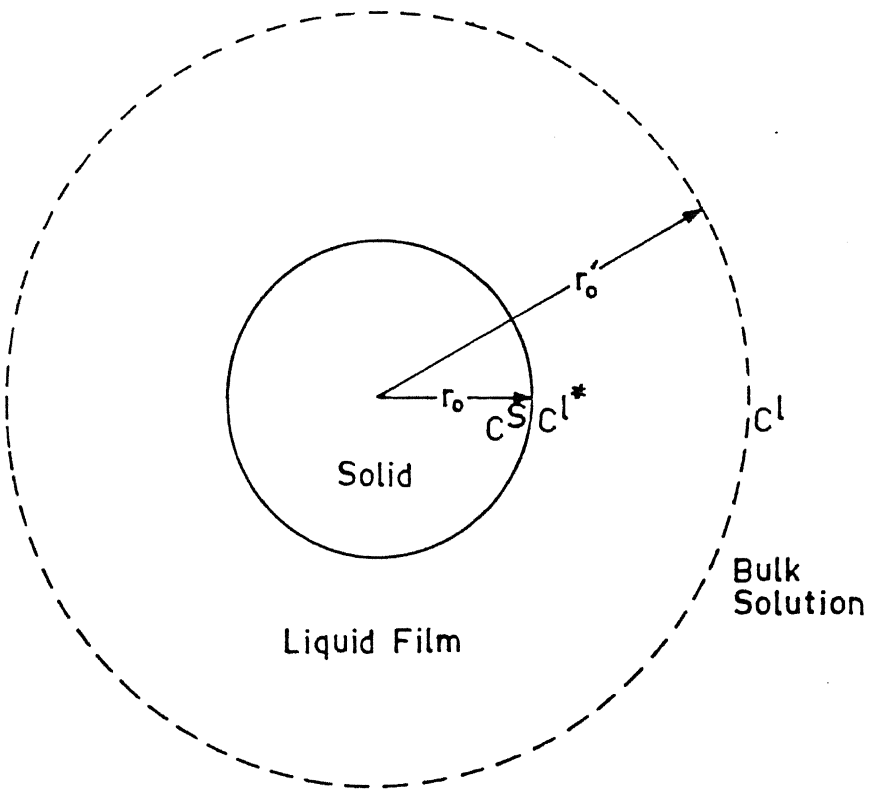


Fig. 5.43. Film Diffusion Theory of Ion Exchange

$$P = -D^{l}(\partial C^{f}/\partial r)_{r=r_o}' \qquad 5.22$$

where D^{l} is the diffusion coefficient in the liquid.

The total rate of flow of adsorbate across the film, dQ/dt, is given by

$$dQ/dt = 4\pi r_o^2 D^{l}(\partial C^{f}/\partial r)_{r=r_o}' \qquad 5.23$$

which for a linear concentration gradient

$$(\partial C^{f}/\partial r) = (C^{l}-C^{f}_{r=r_o})/\Delta r_o \qquad 5.24$$

may be written as

$$dQ/dt = \frac{4\pi r_o^2 D^{l}(C^{l}-C^{f}_{r=r_o})}{\Delta r_o} = \frac{3D^{l}(\frac{4}{3}\pi r_o^3 C^{l}\chi - Q)}{r_o \Delta r_o \chi} \qquad 5.25$$

Defining the constant R by $3D^{l}/r_o\Delta r_o\chi$, and noting that the the total amount adsorbed at equilibrium Q_∞, from a very large amount of solution C^{l} for all time is given by $4\pi r_o^3 C^{l}\chi/3$, it is seen that Equation reduces to

$$dQ/dt = R(Q_\infty - Q) \qquad 5.26$$

which, upon integration for the condition that Q = 0 when t=0, becomes

$$Q = Q_\infty[1-\exp(-Rt)] \qquad 5.27$$

Defining the fractional attainment equilibrium, U (t), by Q/Q_∞, equation 5.27 may be written alternately as

$$\ln [1- U(t)] = -(Rt) \qquad 5.28$$

This equation is exactly same as the one obtained for Helferich model for reversible kinetics, the linearisation of which is presented in Figure 5.42. The model forms the predicted linear fit in the first

fifteen minutes (Figure 5.42). This behavior suggests that the process is limited by film diffusion in the first fifteen minutes of solid-liquid contact. This observation is in harmony with the earlier finding that most of the metal being taken up was by ion exchange process. Ion-exchange is a specific interaction and during the initial period of biosorption this process has to be predominant. The exchange reaction at the site of interaction is so fast that the adsorption is limited by the rate at which adsorbate is being brought to the adsorbent surface (film diffusion).

5.5.2 Interruption Test

The confirmation of rate limiting process in adsorption may be obtained by conducting an interruption test (Helferich, 1962). In an interruption test, the adsorbent is periodically separated from the adsorbate solution for a brief period and then recontacted. When the concentration gradient of adsorbate exists on the adsorbent due to differential rates of film and pore diffusion, this pause is expected to give an opportunity for the concentration gradient to level off. When pore diffusion is rate limiting, the rate of removal will increase immediately after reimmersion as against an uninterrupted sample. However if film diffusion is the rate limiting process, no change in kinetic profile will be observed upon reimmersion.

The kinetic plot of a sample subjected to multiple interruption is presented in Figure 5.44. The kinetic profile of uninterrupted sample is also given for comparison. The curves indicate no increase in uptake rates before and after interruption indicating that film diffusion is rate limiting process. Even after the initial rapid uptake phase, where film diffusion is expectedly rate limiting, pore

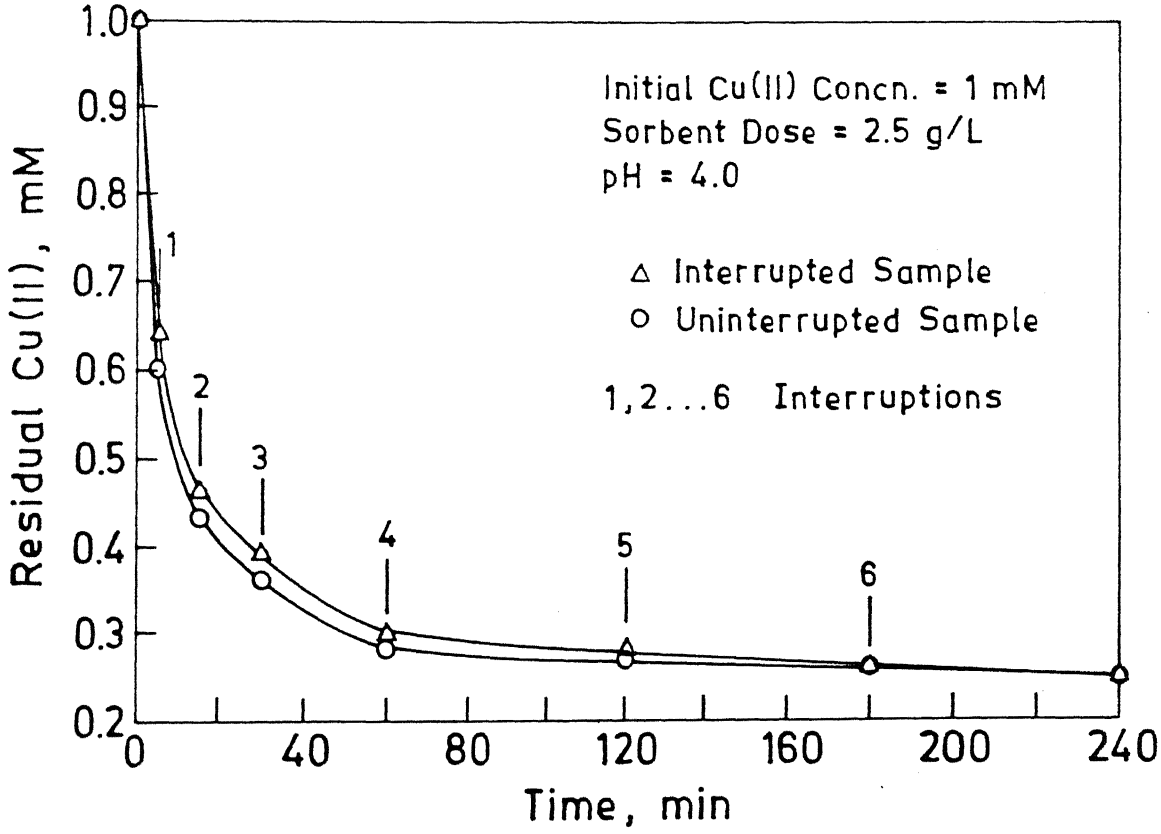


Fig. 5.44. Multiple Interruption of Biosorption and its Effect on Cu(II) Uptake .

diffusion is not the rate limiting process upto 240 minutes. This implies that the slowing down of adsorption rate after 15 minutes is a reflection of not only the lower affinity of the remaining sites for the adsorbates but also the lower concentration gradient available in the liquid phase to effect the mass transfer.

5.6 EFFECT OF AQUEOUS ENVIRONMENT PARAMETERS ON BIOSORPTION

5.6.1 Effect of pH

The pH of the aqueous medium can affect the uptake of metal ions by biosorbents in many ways. At high pH values metals precipitate (vide Figure 2.3) and adsorption is neither possible nor necessary. At lower pH values on the other hand, the concentration of protons are very high that many metal-adsorbent bonds are made labile and the protons effectively compete with metal ions for the binding sites. At intermediate pH values, many species of the metal other than its free ion are formed and the uptake depend upon how these metal species are adsorbed *vis-a-vis* the metal ions.

In the case of ion exchange type of adsorption, the competition of protons is the most significant factor. Ion exchange resins are generally regenerated by treating the exhausted bed with mineral acid and hence at low pH values the uptake of copper obviously will be minimum or totally absent. The copper (II) uptake at various pH values by *G. lucidum* is presented in Figure 5.45. Percentage metal uptake increased from 0.2 % at pH 2 to 76.6 % at pH 6, beyond which experiments were not conducted as the copper would start precipitating. These observations are in accordance with the proposed hypothesis of biosorption.

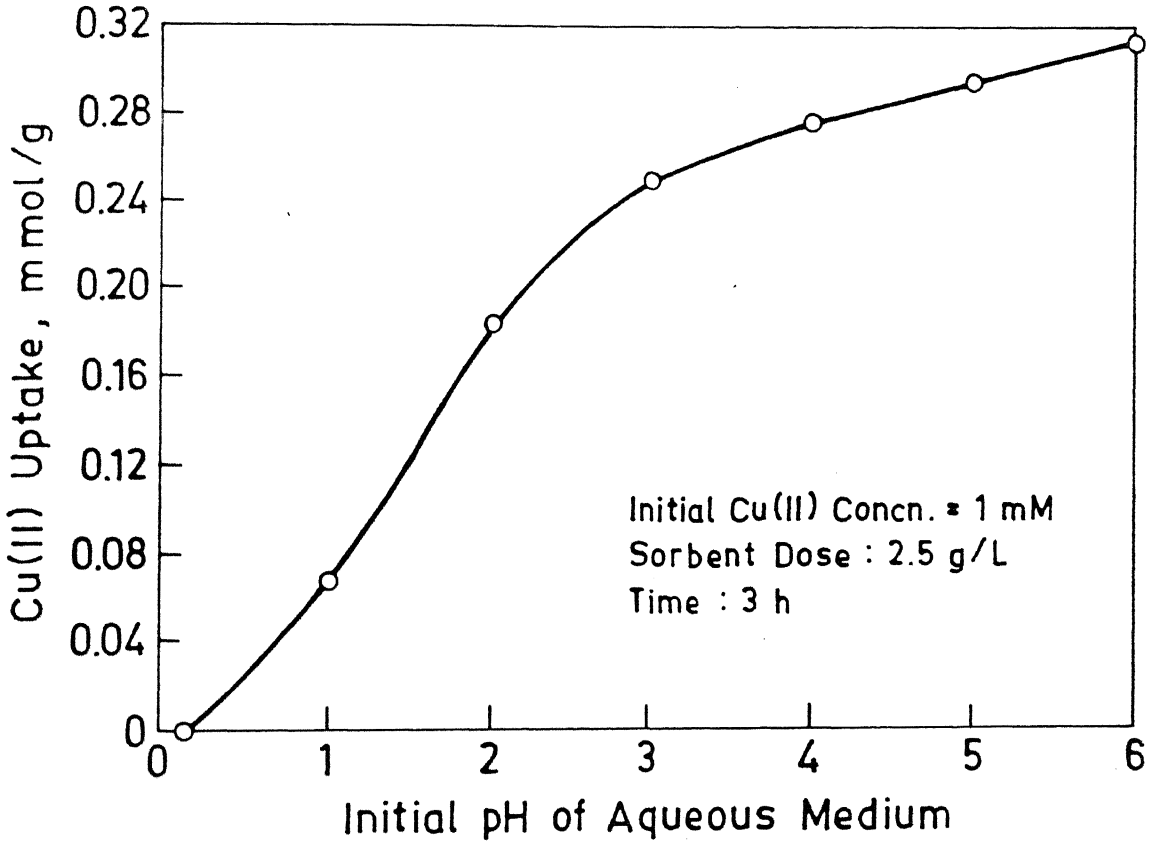


Fig. 5.45. Effect of pH of the Adsorbate Solution on Biosorption of Cu(II).

5.6.2 Effect of Anionic Ligands

Metals are not found in isolation in the environment. A number of anionic ligands are often found along with metals in industrial waste waters in real-life situations. These anionic ligands form a large number of species with the metal, and the resultant species may be (i) strongly adsorbable (ii) weakly adsorbable and (iii) non-adsorbable, depending upon the nature of ligands and the biosorbent. The stability constants of soluble ligangds and the biosorbent with the metal dictate the distribution of metal between these two phases. As it is not possible to theoretically evaluate the metal-biosorbent stability constants, one has to resort to experiments to determine the effects of various anionic ligands on the uptake of copper by *G. lucidum*.

A total of ten ligands commonly encountered in metal processing effluent streams were used in the present study. To obtain the effect over a broad spectrum of ligand concentrations, two sets of experiments with 1 and 10 mM of ligand concentration were performed. Wherever possible sodium was kept as the cationic component to minimise the influence of cation concentration on the process. Since copper sulphate was the salt employed for maintaining the copper (II) concentration, the blank solution for 10 mM was adjusted by sodium sulphate.

The effect of various ligands on biosorption of copper by *G. lucidum* is presented in Figure 5.46. Even at 1 mM concentration, cyanide substantially inhibited copper (II) uptake by *G. lucidum*. EDTA also inhibited metal uptake substantially. The inhibition by other ligands followed the order Perchlorate› Persulfate › Tartrate› Citrate›

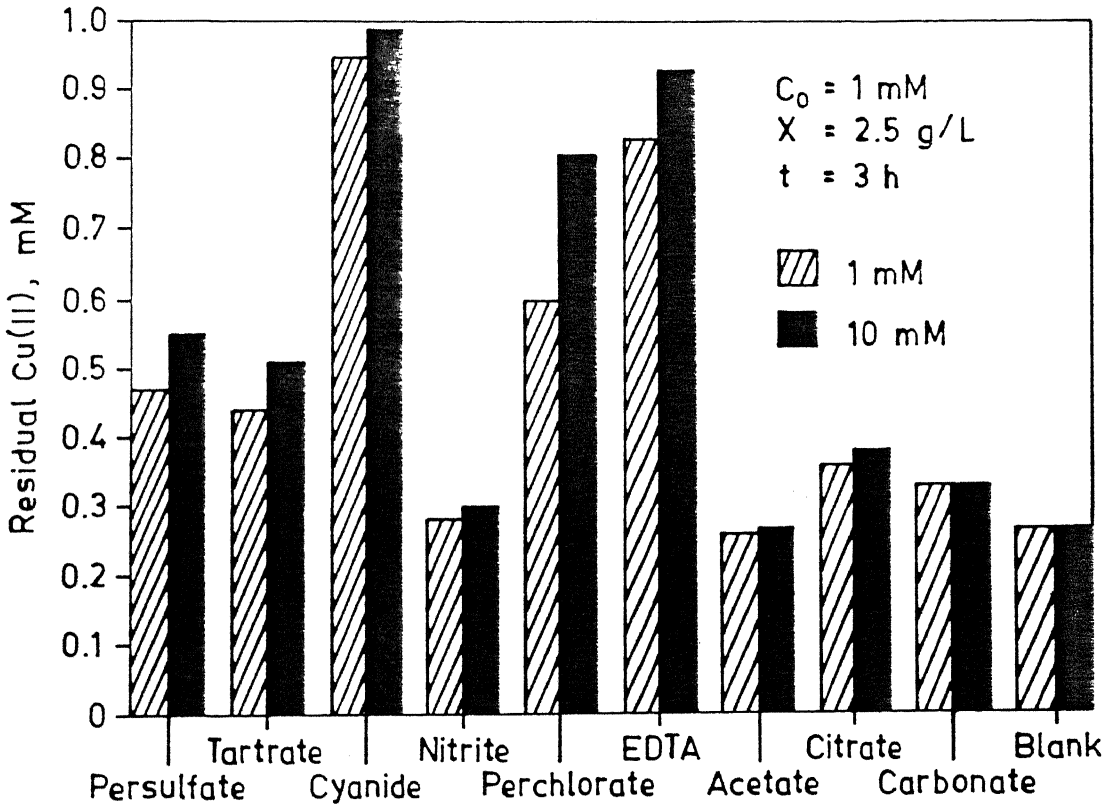


Fig. 5.46. Effect of Anionic Ligands on Cu(II) Uptake.

Carbonate> Nitrite> Acetate. Influence of anions on uptake of metallic cations La^{3+},Cd^{2+},Pb^{2+},UO_2^{2+} and Ag^{+} by *R. arrhizus* were reported by Tobin and others (1987). They also reported significant detrimental impact of EDTA on biosorption. The adverse impact of cyanide on cadmium uptake by *Citrobactor* sp. immobilised on gel and solid supports was reported by Macaskie and others (1987). *G. lucidum* has exhibited similar response to EDTA and cyanide as *Rhizopus arrhizus* and *Citrobactor sp.*

5.7 DESORPTION STUDIES

The feasibility of recovering the sorbed metal has been one of the advantages of biosorption process as against precipitation techniques. The knowledge regarding metal coordinating environment is expected to aid designing the desorption process based upon sound theoretical understanding. Chemically coordinated metal may be eluted from the adsorbent by four different processes, ie.,

1. If ion exchange is the mode of adsorption, then eluting and regenerating the exhausted sorbent using a low cost and less polluting ion other than proton at higher concentration is a feasible proposal
2. In case metal get exchanged with protons, the exhausted adsorbent can be regenerated by applying high concentration of H^{+} ions. A high concentration of H^{+}ions also make most metal complexes labile and thus acidic treatment can be employed to elute metal ions even when the metal exchange is not with protons.

3. Employing a liquid phase complexing agent like EDTA which has a higher stability constant for the adsorbed metal than the adsorbent, the metal can be eluted.

4. Destructive digestion can recover all the metal adsorbed irrespective of the uptake mechanism, however, the sorbent cannot be reused.

In the present study, 0.1 molar solutions of calcium chloride, hydrochloric acid and EDTA were employed as eluents. A reaction mixture maintained at at pH 4.0 as in the case of adsorption solution but without copper was also used as eluent. Biosorbent *G. lucidum* after copper (II) adsorption was agitated with these eluents and after the stipulated reaction time, the adsorbent was separated and copper was analysed in the supernatant. The results are presented in Figure 5.47. Complete recovery of metal was possible with both EDTA and HCl, while calcium chloride yielded 30 % elution at similar molarities. The desorption using distilled water maintained at pH 4.0 was, however, negligible reinforcing the observations regarding the strong complexation between the biosorbent and copper.

The information about the efficiency of complexing agents to elute metal from adsorbents can be put to effective use in biosorption. If this process is to be employed for any element other than copper, ligands with high selectivity for that metal should be used for desorbing the metal and reusing the sorbent. The information about metal ligand selectivity is well documented in literature. While directing this process for the multi metal system, selective elution can be achieved by careful selection of complexing agents. Such an approach has been suggested by Darnall and others (1986), wherein

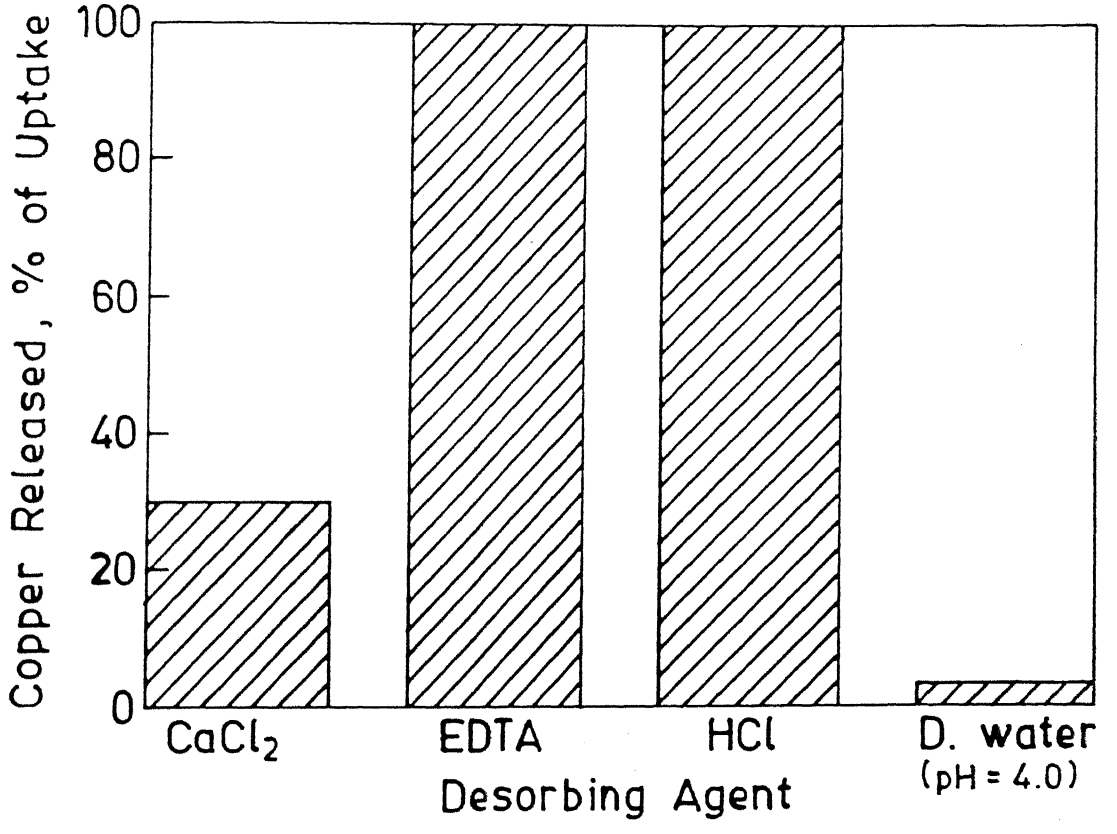


Fig. 5.47. Performance of Various Eluents for Desorption of Cu(II) from G.lucidum.

different metals were selectively eluted from algae, *Chlorella vulgaris*.

In contrast to complexing agents for desorption, the use of protons for desorption is a non-selective process and is a function of the hydrogen tension in the aqueous medium. The optimum pH can be determined by gradually lowering the pH of the aqueous medium with the subsequent monitoring of the metal concentration in the medium. The desorption of Cu(II) from *G. lucidum* by mineral acid is presented in Figure 5.48. The biosorbent was separated from the adsorption medium after metal uptake, washed and was resuspended in triple distilled water. The pH of this reaction mixture was gradually brought down and the copper concentration in the aqueous phase corresponding to different pH values were measured. At pH 5 only an insignificant portion (3%) of metal was desorbed and as the pH was brought down to 1.5 by addition of 1 N hydrochloric acid, over 90 % metal which was originally sorbed by the biosorbent was released into the solution.

It can be observed that at pH below 1.5 more than 80 % of all adsorbed metal have been desorbed. Also combining this observation with the near total desorption with 0.1 molar HCl, a normality of 0.1 N was found to be suitable to elute biosorbed metals from the *G. lucidum*. Kinetics of desorption was performed using this acid and the results are presented in Figure 5.49. It can easily be seen that desorption is an extremely rapid process with more than 90 % desorption being achieved in first 15 minutes and complete desorption in an hour.

5.8 UPTAKE OF OTHER METALS BY BIOSORPTION

Lab grown biosorbents reported thus far do not exhibit specificity and hence have been employed for the uptake of a wide range

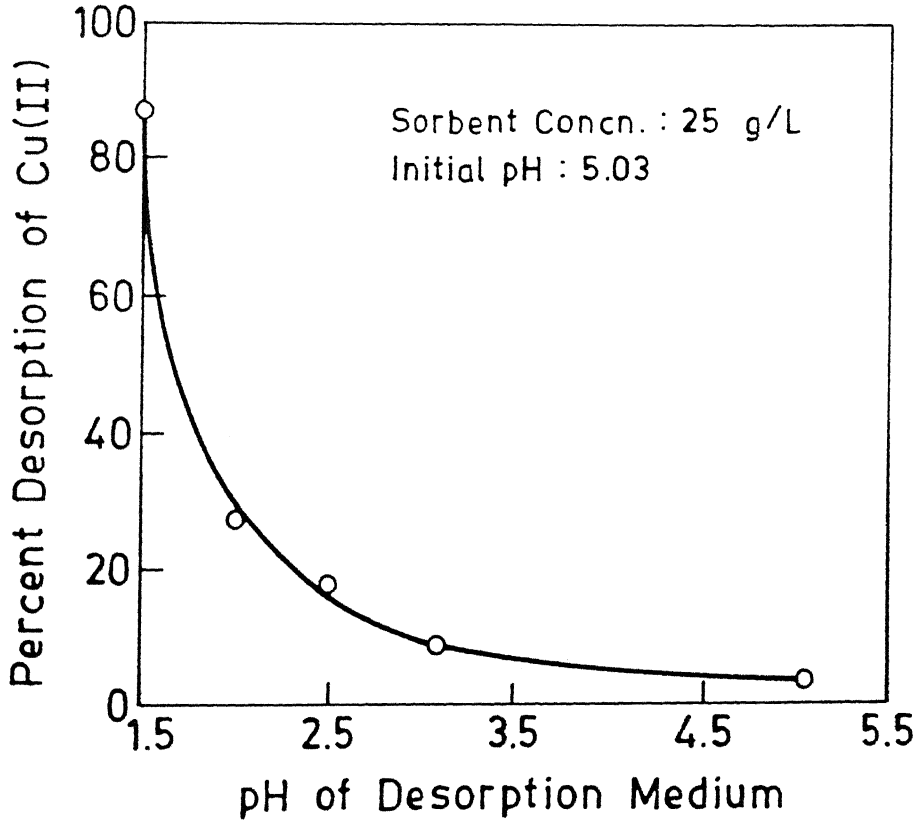


Fig. 5.48. Desorption of Cu(II) into Aqueuos Phase at Different pH Values.

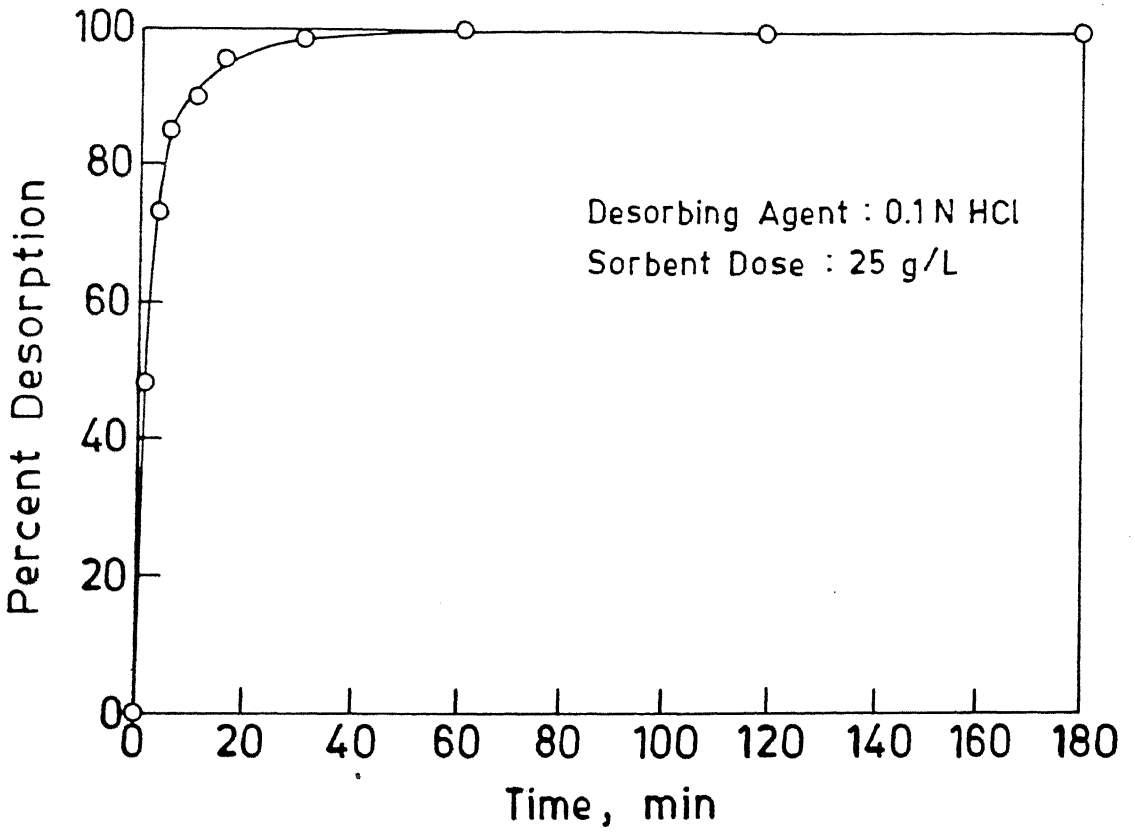


Fig. 5.49. Kinetics of Desorption of Copper (II) from G.lucidum.

of metals (Muzzareli, 1980) This versatility is a recognized advantage of biosorption process and selectivity, if necessary, is achieved at the desorption stage using selective eluting agents (Volesky, 1990).

The uptake potential of *G. lucidum* for Chromium (III), Manganese (II), Cobalt (II), Nickel (II), Copper (II), Zinc (II), Cadmium (II) and Mercury (II) were evaluated. These metals were selected to obtain a representative sample from the broad spectrum of metals in the environment. The uptake of these metals were evaluated under identical environmental conditions and the maximum uptake capacity was calculated using Langmuir's isotherm. The Q_{max} values presented in Table 5.7.

Table 5.7 Uptake Capacity of Biosorbent M_1 for Different Metals

Metal	Uptake Capacity Q_{max} (mmol/g)
Cr	0.33
Mn	0.36
Co	0.31
Ni	0.29
Cu	0.38
Zn	0.31
Cd	0.26
Hg	0.26

It can be seen from the values presented that all metals studied were taken up by the biosorbent. The minimum uptake was observed for mercury and cadmium (0.26 mmol/g) while maximum uptake capacity was observed for copper (0.38 mmol/g). The metal uptake values followed the the sequence Cu > Mn > Cr > Co = Zn > Ni > Cd = Hg. Many researchers have proposed correlation models between the maximum metal uptake potential with the ionic radii (Tobin and others, 1984) or the ionic potential (Volesky, 1990). A plot of Q_{max} against ionic radii and ionic potential is presented in Figures 5.50 (a) and (b) and it can be observed that there is no trend between the variables. Hence further analysis of these data was not attempted.

5.8.1. Metal Uptake As a Function of Donor Site Preferences

A biologically significant classification of metals was presented by Ahrland and other (1958). They examined the trends in the magnitude of equilibrium constants that describe the formation of metal-ion and ligand complexes and indicated that metals could be separated into three categories. In general terms, this reaction and the corresponding equilibrium constant are defined as follows:

$$M + L \longrightarrow ML \qquad 5.29$$

$$K_{ML} = [ML]/[M][L] \qquad 5.30$$

here M represent the metal ion, L the ligand, ML the metal-ligand complex and K_{ML} the stability constant, the square brackets denoting the molar concentration for aqueous solutions. The authors classified metals into Class A, Class B and Borderline elements based upon the values of K_{ML} for different metal-ligand systems.

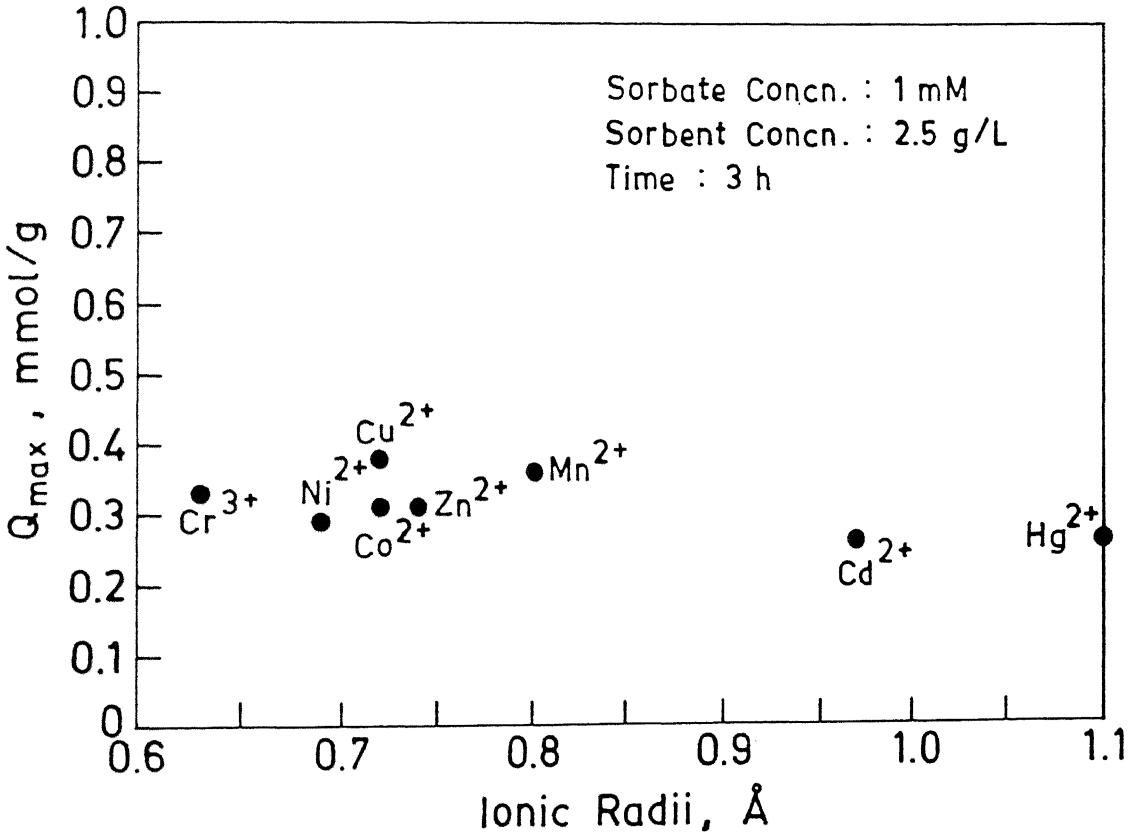


Fig. 5.50a. Relation between the Ionic Radii of Elements and Maximum Specific Uptake by Biosorbent M_1.

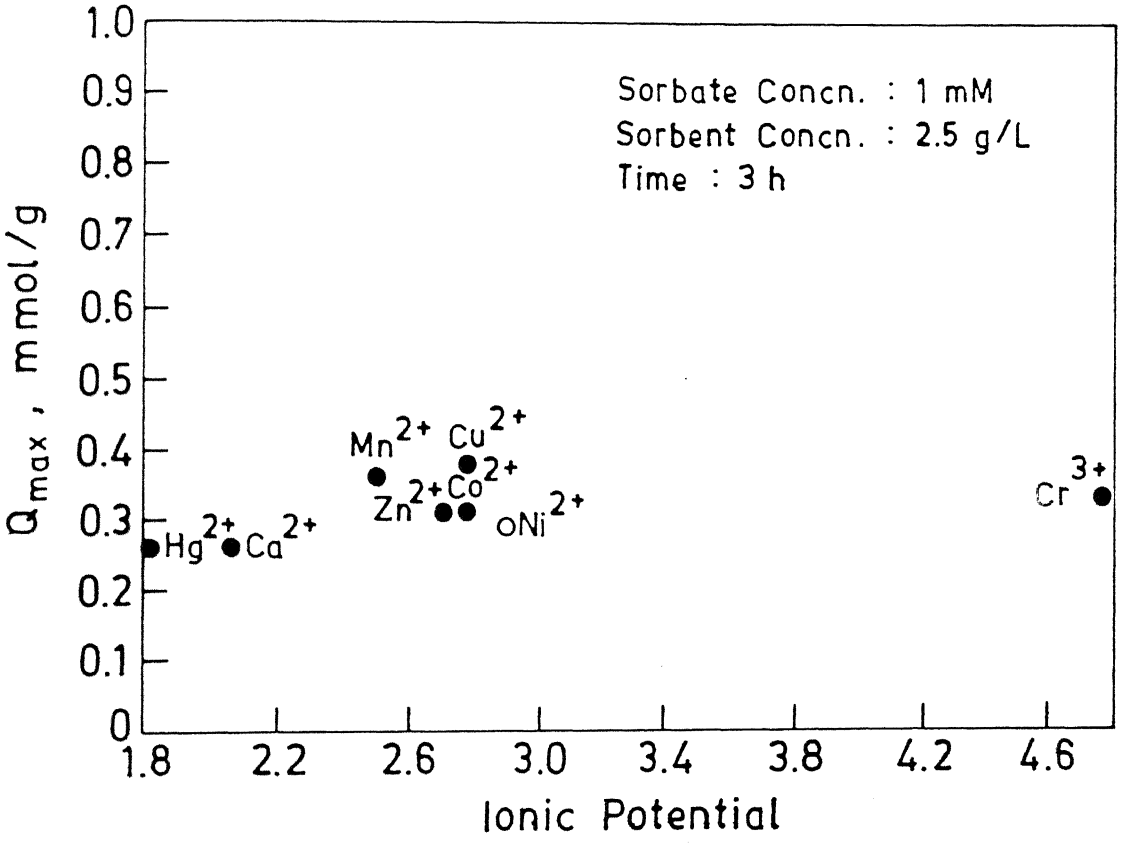


Fig. 5.50b. Relation Between the Ionic Potential of Elements and Maximum Specific Uptake by Biosorbent M_1.

Class A metals were defined as those, on the basis of the equilibrium constants, have the following ligand or donor atom preference sequence for ligands

$$F^- > Cl^- > Br^- > I^-$$

and for metal binding donor atoms

$$O > N > S$$

In contrast, class B metal ions exhibit the opposite preference sequences:

$$I^- > Br^- > Cl^- > F^-$$

and for donor atoms

$$\text{and } S > N > O$$

The borderline metal ions form an intermediate group which is ambivalent in that these ions exhibit a catholic affinity for the above metal binding donor atoms and ligands. The detailed classification of the important elements is presented in Table 5.8. Among the borderline ions, Class B character increases in the order $Mn^{2+} < Zn^{2+} < Ni^{2+} < Co^{2+} < Cu^{2+} < Cd^{2+}$.

It may be relevant here to mention that in the biological environment mercury is a sulphur seeking element while copper shows equal preference to nitrogen and oxygen. Calcium, which is being exchanged for the adsorbate metal ions, is an oxygen seeking element. Lower uptake capacity of the biosorbent *G. lucidum* for sulfur seeking element mercury is probably due to the presence of oxygen dominated donor sites which exhibit low affinity for sulfur seeking elements. Cadmium, which among the borderline elements shows maximum preference for sulfur sites is also taken up only to a lower degree as compared to

Table 5.8. Classification of Metal Ions Based on Donor Site Preferences*

Category	Metal ions
Class A O > N > S	Li^{+}, Be^{++}, Na^{+}, Mg^{++}, K^{+}, Ca^{++}, Sc^{+++}, Sr^{++}, Y^{+++}, Cs^{+}, Ba^{++}, Al^{+++}, Lanthanides, Actinides
Borderline	Ti^{++}, V^{++}, Cr^{+++}, Mn^{++}, Fe^{++}, Co^{++}, Ni^{++}, Zn^{++}, Cd^{++}, Ga^{+++}, In^{+++}, Sn^{++}, Cu^{++}
Class B S > N > O	Pd^{++}, Pt^{++}, Ag^{+}, Au^{+}, Hg^{++}, Tl^{+++}, Pb^{4+}, Bi^{+++}

* Ahrland and others, 1958

other borderline elements. It may be cited here that Cadmium adsorbed onto algal biomass *Chlorella vulgaris* could only be eluted by thiourea indicating the strong preference of this ion for sulfur (Darnall and others, 1986). In the case of manganese, the higher uptake can be attributed to its preferential interaction with oxygen (Manganese exhibits maximum class A characteristics among the borderline elements). Uptake capacity for other elements also broadly follow the classification.

The uptake capacity for copper is the highest among the metals investigated. This possibly is a result of the unique positioning of copper, in that it shows equal affinity for both oxygen and nitrogen donor sites (Sengupta and others, 1991). This is further substantiated by the fact that copper ion is being coordinated to both oxygen and nitrogen donor atoms of *G. lucidum* as indicated by the EPR studies.

This probably results in the availability of more donor sites to copper as compared to purely oxygen seeking or nitrogen seeking elements.

The above observations regarding the uptake capacity and affinity of donor sites on biosorbent for different class of elements was experimentally verified by conducting metal uptake studies using mixed metal systems. The adsorbate ions studied are lanthanum (Strictly Class A) and Copper (Borderline). When metal uptake studies from a mixture of these elements (Containing 1 mM each) was conducted, lanthanum was taken up preferentially to copper by *G. lucidum*. However both elements were taken up when studies were conducted independently (Table 5.9).

Table 5.9 Preferntial Adsorption of Elements by Biosorbent M_1

Elements Present	% Uptake	
	La	Cu
Cu	-	73.5
La	90.00	-
Cu+La	57.00	28.00

The practical significance of the observed biosorption potential of *G. lucidum* is substantial. The biosorbent can be directed for the uptake and concentration of any of the metals most of which has been classified as hazardous. In the case of industrial units processing more than one of these elements, a compact treatment unit employing *G. lucidum* would offer an effective pollution control option because it exhibits both versatility and selectivity.

5.9 BIOSORPTION OF RARE EARTH ELEMENTS

The studies on field applications of the biosorbent *G. lucidum* was conducted using rare earth processing industry as an example. This is based on the following scientific and practical reasons.

1. The mechanism studies identified calcium to be exchanging ion for the biosorbent and hence it was hypothesised that the site of metal coordination is oxygen dominating. Rare earth ions are oxygen seeking like calcium and hence it was expected that the biosorbent with an oxygen donor site will be successful in binding these elements.
2. Rare earth elements are widely employed as calcium probes in bioinorganic chemistry (Prados and others, 1974).
3. *G. lucidum* has a capacity for taking up a wide spectrum of metals and since rare earths being a mixture of elements, they can be expected to be effectively handled by biosorbents.
4. The process of ion exchange is widely employed for concentration and separation of rare earths. The technology shock while implementing biosorption and the resistance to acceptance will be minimal.

Because of their electronic configuration, rare earth elements have closely resembling characteristics. In the present investigation, preliminary studies are hence conducted with a model element lanthanum and a mixture of all rare earths. Uptake studies were, however, conducted with pure elements Nd^{3+}, Pr^{3+}, Dy^{3+}, Gd^{3+}, Tb^{3+}, Y^{3+}, Eu^{3+} and Sm^{3+} also.

5.9.1 Kinetics of Rare Earths Uptake:

The rate of uptake of rare earths was evaluated using lanthanum and mixed rare earth chlorides. The results are presented in Figure 5.51. The rate of uptake is very rapid for both but, better in the case of mixed rare earths with the equilibrium being established in only thirty minutes. In the case of lanthanum, there was a very rapid uptake for the first fifteen minutes with apparent equilibrium being established by one hour. The kinetic variation of the pH of the aqueous medium is presented in Figure 5.52. The pH drops from 5.86 to 4.90 (a proton equivalent of 0.011 mM) in first fifteen minutes in the case of lanthanum and from 6.44 to 5.3 (proton equivalent 0.007 mM) in the case of mixed rare earths. The change in pH is insignificant to suggest the exchange of La and mixed rare earth with protons. Hence the observed high removal of rare earths might be due to exchange with other ions. The information on this is presented in succeeding sections.

5.9.2 Mode of Uptake of Rare Earths

The mechanism of uptake of rare earths is expected to be similar to that of other heavy metals presented, because of the chemical similarities. The preferential uptake of lanthanum to the binding sites was also described in section 5.8. The mechanism of metal uptake was further verified by employing instrumental techniques.

Rare earths are also paramagnetic species and hence EPR spectroscopy can be conveniently utilized to verify whether the metal uptake is by physisorption or chemical coordination. Although all rare earths except cerium are paramagnetic, the EPR response of only one of the elements is presented as a model. In this context dysprosium which has the maximum of five unpaired electrons was used and the EPR signal

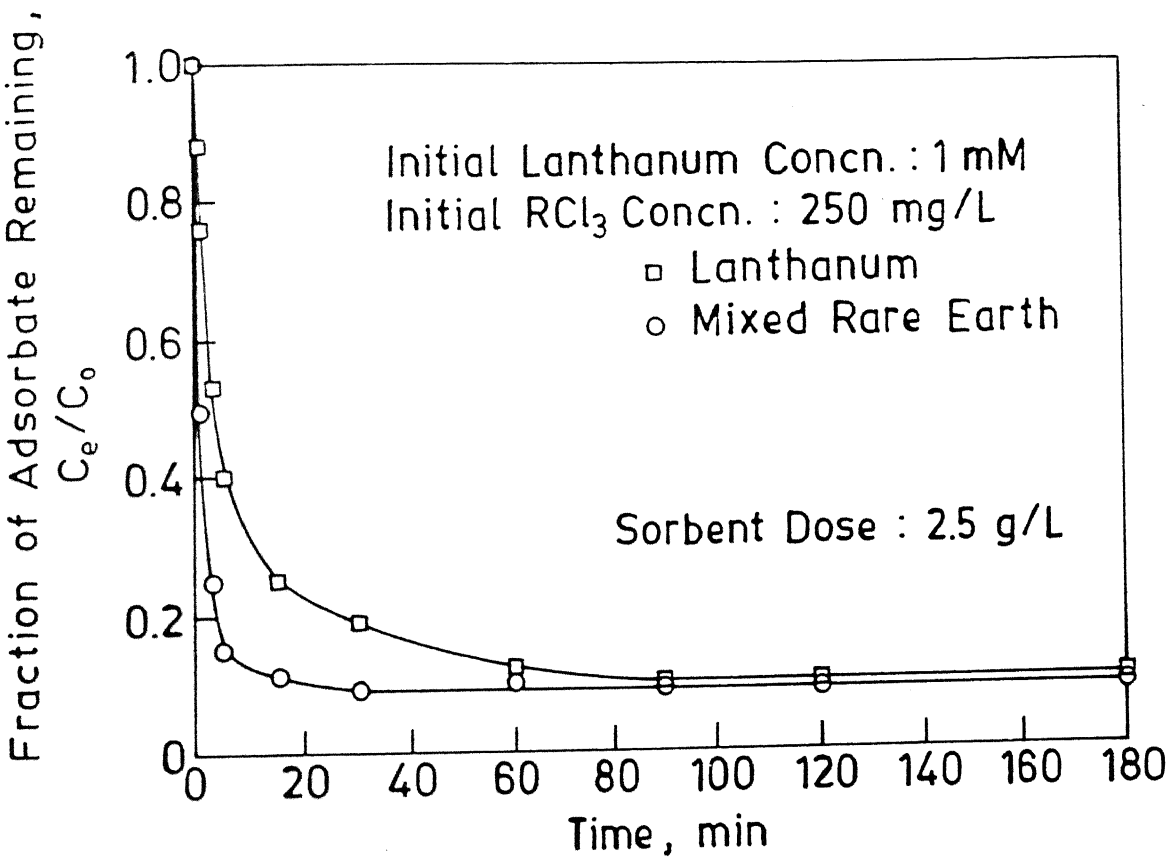


Fig. 5.51. Kinetics of Rare Earth Adsorption.

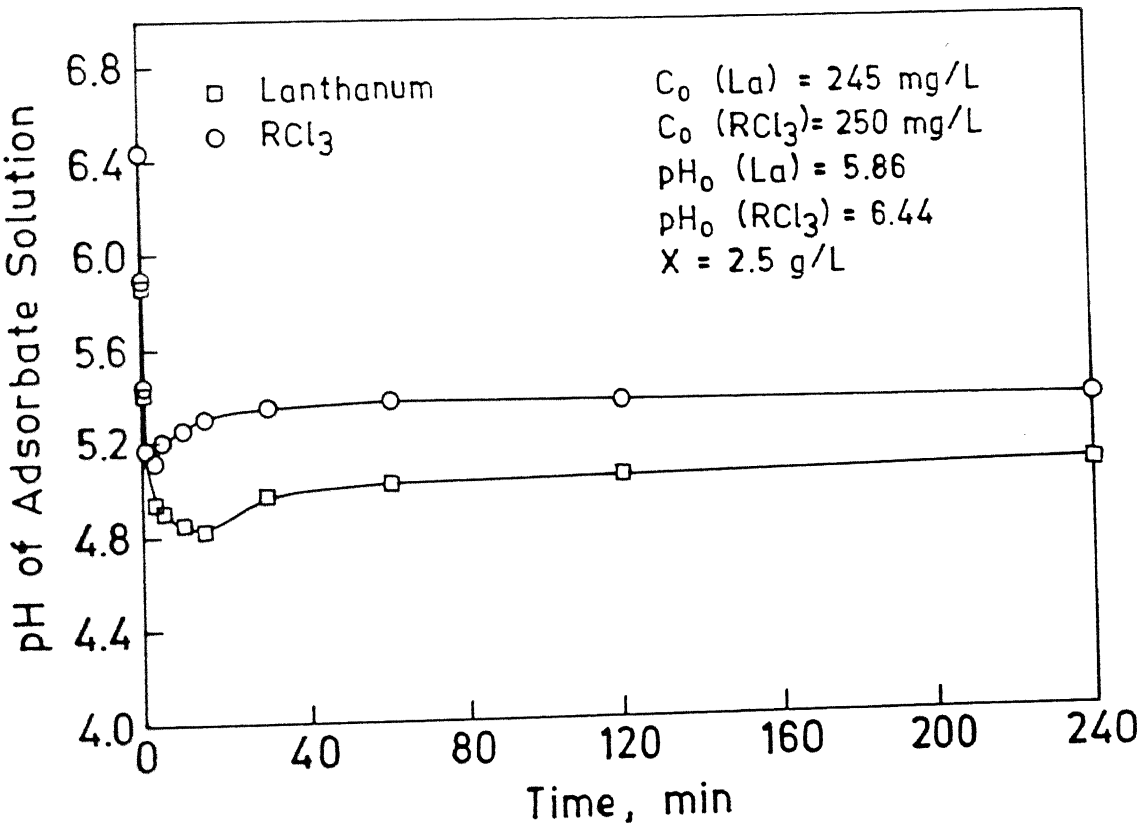


Fig. 5.52. Effect of Biosorption of Rare Earth Element on pH.

of *G. lucidum* after adsorbing dysprosium onto it was obtained and is presented in Figure 5.53. EPR spectra of dysprosium is characterized by multiplet signals due to the number of unpaired electrons, its interaction with nuclei and also its interaction with the coordinating atoms (Taylor, 1975). The EPR of Dysprosium was also characterized by an extremely high g value (7.55) when the spectra was taken in a copper single crystal (Altschuler and others, 1977). In the present study also, the EPR signal is characterized by the multiplet signals with a g value of 6.46. The shift in the signal reflected by the change in g value is indicative of the change in coordinating environment as compared to the studies reported. The EPR signal clearly indicates that the element is chemically coordinated onto the biosorbent, though no further information regarding the coordinating environment could be obtained.

5.9.3 EDAX Studies of Biosorption of Lanthanum by *G. lucidum*

EDAX analysis of the biosorbent after biosorption of lanthanum was also conducted and the resulting spectrum is given in Figure 5.54. A comparison of this with Figure 5.39 indicate the decreased dominance of calcium signal in the biosorbent after adsorption with the distinctive lanthanum signal, suggesting exchange of calcium ions with lanthanum.

5.9.4 Equilibrium with Other Rare Earth Elements

Adsorption equilibrium experiments were conducted with individual rare earth ions to find out their affinity for the biosorbents and maximum uptake potential.

The binding reaction between metal (HM) in aqueous phase and free binding sites on the biosorbent (BS) can be expressed as

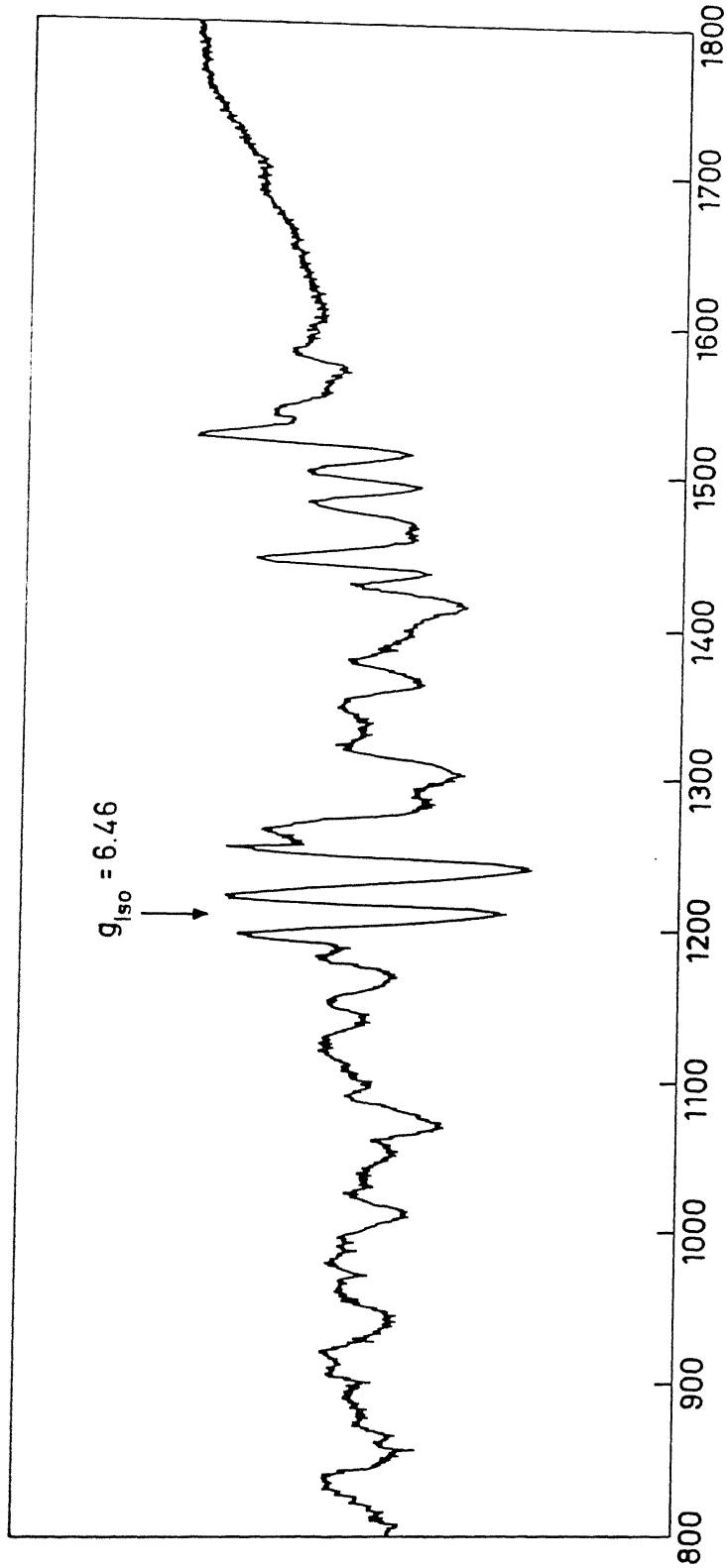


Fig. 5.53. Typical EPR Spectrum of Biosorbent, M_1 After Adsorption of Rare Earth Element Dyspronsium.
[Settings : Modulation = 10 G, Gain = 2×10^4, Mod. Frequency = 100 K, Temp. = 25 °C, Microwave Frequency = 9.21]

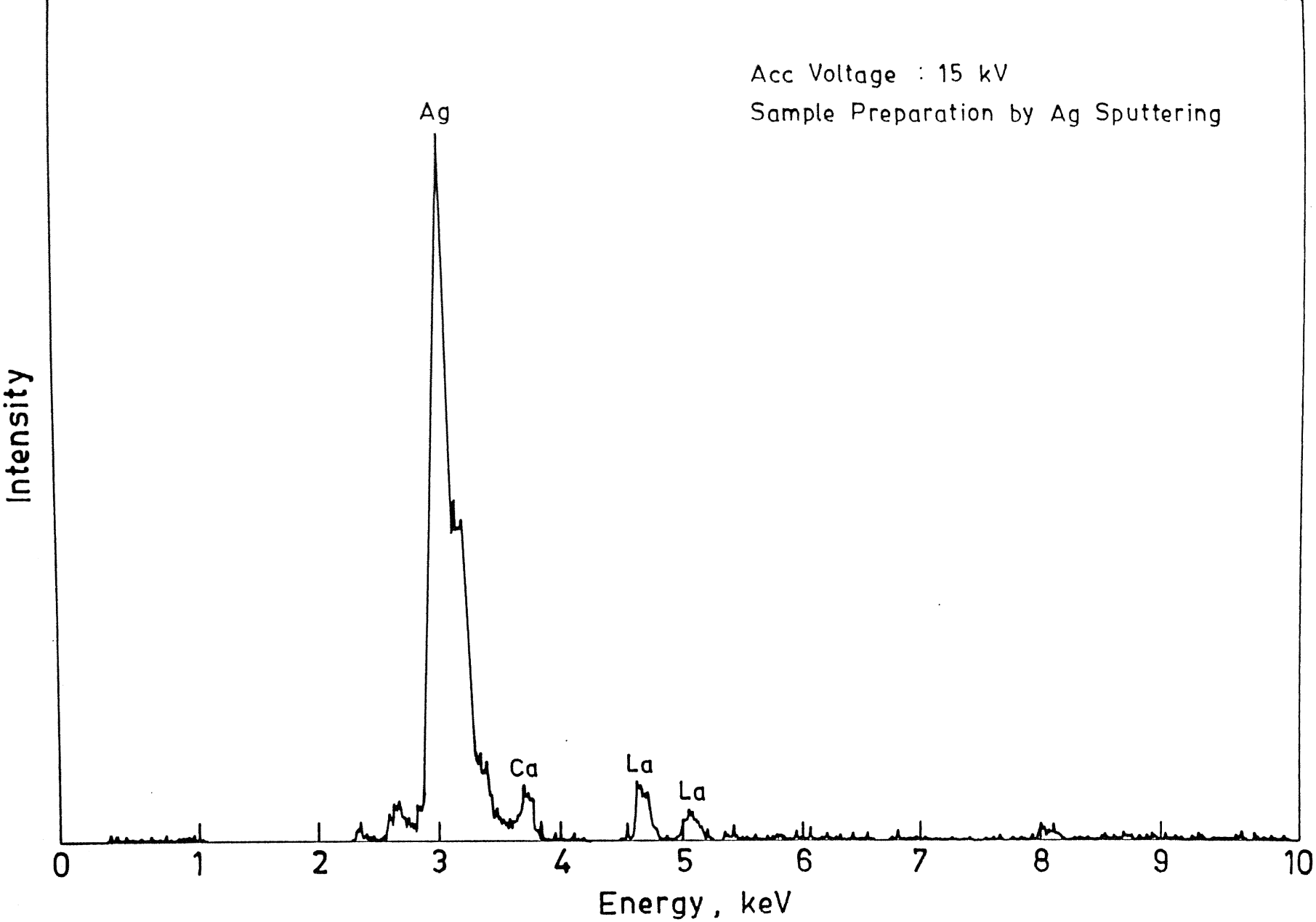


Fig. 5.54. EDAX Spectrum of Biosorbent, M_1 After Lanthanum Uptake.

$$HM + BS \rightleftharpoons HMBS \qquad 5.31$$

where HMBS is the complex formed between the adsorbate and the biosorbent. The conditional stability constant K_c under a given set of environmental conditions (pH, ionic strength and temperature) is defined as

$$K_c = \frac{[HMBS]}{[HM][BS]} \qquad 5.32$$

where [HM] is the concentration of unbound metal in bulk solution, [HMBS] the concentration of metals bound onto the biosorbent and [BS] the unoccupied sites on the biosorbent. As no distinction can be made between different mechanisms for the uptake by the biosorbent, the stability constant reflects a measure of the overall affinity of the biosorbent for the metal.

If Q_{max} is the saturation metal uptake capacity corresponding to maximum number of sites of the biosorbent as defined by the Langmuir theory, then

at any time

$$Q_{max} = [BS] + [HMBS] \qquad 5.33$$

If

C_o = initial concentration of metal in aqueous phase, in mM

and

C_t = Concentration at time t, mM

X = weight of biosorbent, g

then metal bound to biosorbent is given by

$$[HMBS] = q = (C_o - C_t)/X \qquad 5.34$$

$$K_c = \frac{q}{[C_t][Q_{max} - q]} \qquad 5.35$$

$$K_c C_t Q_{max} - K_c q C_t = q \qquad 5.36$$

dividing by $K_c Q_{max} q$

$$\frac{C_t}{q} = \frac{1}{K_c Q_{max}} + \frac{C_t}{Q_{max}} \qquad 5.37$$

The values of conditional stability constant (K_c) and maximum uptake capacity (Q_{max}) can be determined from the slope and intercept of plot of C_t/q Vs C_t. This equation is similar to linearised Langmuir equation 5.9 with 'b' equivalent to K_c (Ruzic, 1982). This stability constant can be used as a parameter for comparison when different metals are adsorbed under identical conditions.

The stability constant (K_c) and the maximum uptake potential as evaluated by the above technique is presented in Table 5.10. The corresponding values for copper is also included for comparison. All rare earth elements have a stability constant (a measure of affinity) far in excess of that of copper (II), which is explainable in light of the preference shown by oxygen environments to rare earth elements over copper.

Table 5. 10 Maximum Uptake Capacity (Q_{max}) and Stability Constants (K_C) of Rare Earths and Copper by Biosorbent M_1

Element	Q_{max} (mmol/g)	K_C (L/mM)
La	0.36	19.89
Pr	0.32	31.57
Nd	0.30	32.82
Sm	0.33	23.89
Eu	0.33	17.21
Gd	0.33	26.33
Dy	0.30	28.24
Cu	0.38	12.65

5.9.5 Column Studies

Adsorbents are generally contacted with adsorbates in packed bed reactors. Same is the case with ion exchange resins, where down flow packed bed reactors are the most common configuration. In view of the promising nature of *G. lucidum*, it was considered appropriate to conduct bench scale studies with packed bed reactors in the down flow mode to evolve design criteria for the development of prototype.

5.9.5.1 Hydraulic Loading and Head Loss

In addition to the information on uptake potential, possibility of metal recovery and reuse of adsorbent bed, data regarding the operation of the fixed bed reactor in terms of the hydraulic loading rate and development of head loss is also essential. Many biosorbents, which gave excellent uptake potential in batch

reactors had to be immobilized or complicated reactor configurations like pulse bed reactors had to be employed (Volesky, 1987) as they were not amenable for the fixed bed operation. In order to evaluate the behavior of *G. lucidum* for continuous flow operation, studies were conducted using fixed bed reactor.

The optimum flow rate necessary for best performance of the adsorption bed depends on the rate of uptake of the adsorbate from wastewater by the biosorbent. Both theoretical and experimental data support the contention that there are critical velocities for liquids passing through the porous bed which change the nature of the resistance to diffusion. At low velocities, the solute content of the stagnant film surrounding the adsorbent will be depleted more rapidly than can be replaced by diffusion from the main body of the liquid. As the velocity is increased, a stage will be reached where the controlling rate will be the ability of the adsorbent to take up the adsorbate as rapidly as it is transported to the surface from the bulk liquid.

Column studies were conducted using the experimental set up presented in Figure 4.2 with varying bed depths and flow rates to evaluate the head loss profile within the biosorbent bed. Tap water at flow rates varying from 3 $m^3/m^2/h$ to 17 $m^3/m^2/h$ was passed through the column at a fixed static head of 150 mm above the to of the column. Head loss values were measured at bed depths 150, 300, 450 and 600 mm. The head loss profile as found out by experimentation is presented in Figure 5.55. At flow rates suggested by EPA design manual for activated carbon adsorption (EPA, 1973) ie., <3.5 $m^3/m^2/h$, the head loss developed is only nominal. The head loss increased with the

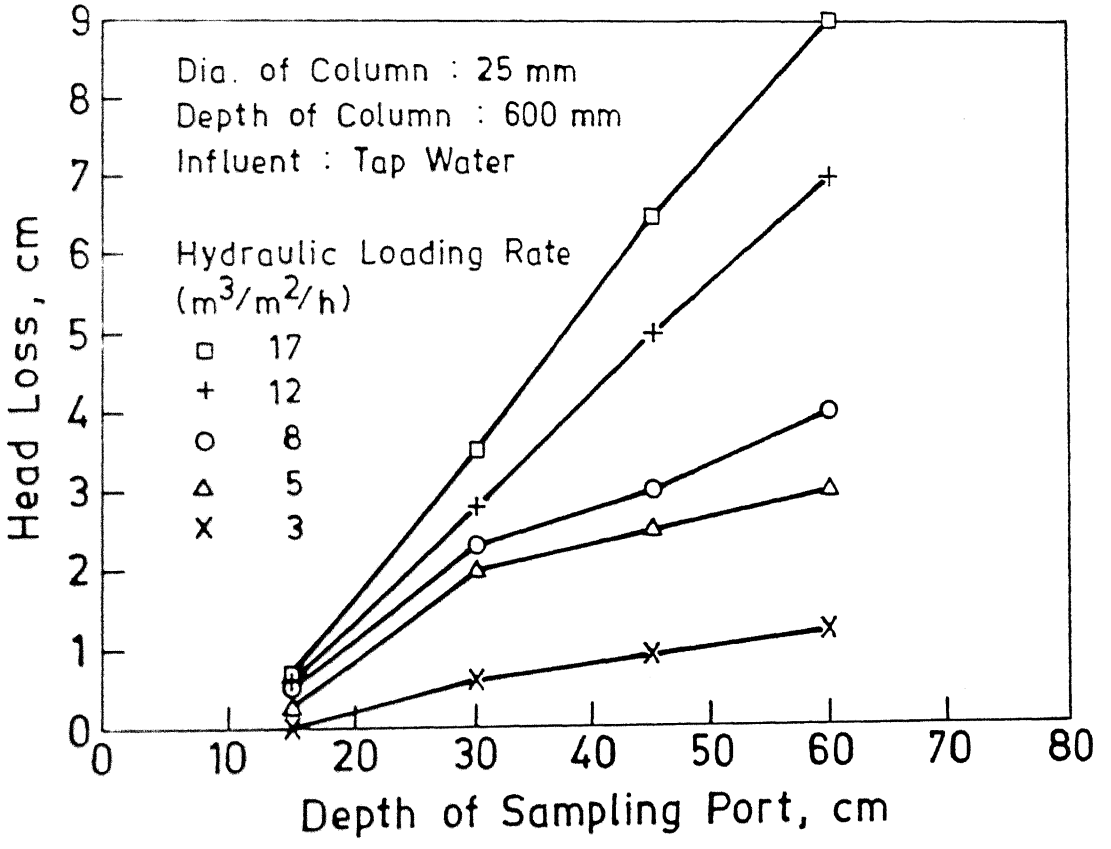


Fig. 5.55. Head Loss in a Downflow Packed Bed Adsorption Reactor.

increase in hydraulic loading rate. The experimental results indicate that within hydraulic flow rates usually encountered, the development of head loss is very gradual in columns packed with *G. lucidum*.

5.9.5.2 Attrition of Biosorbents in Packed Bed Reactors

During the fixed bed operation, sorbent is subjected to extensive static and dynamic pressures. It is, therefore, important to evaluate how effectively the biosorbent withstand this pressure. The physical breakdown of the biosorbent as a percentage of initial weight was found to be only 0.85 % at a very high hydraulic flow rate of 36 $m^3/m^2/h$, indicating that the biosorbent to be highly suitable for use in a packed bed reactor.

5.9.5.3 Recovery of Rare Earths and Reuse of Column

Preliminary studies were conducted using a column 25 mm diameter and 600 mm long. The column was packed with biosorbent and was challenged with a mixed rare earth chloride solution, buffered at pH 4.0 by 0.1 M acetate buffer. The column run was continued upto complete exhaustion. Once the column was exhausted, it was regenerated by 0.1 N HCl and the volume needed was 1/40th the throughput (Volume of effluent treated). After desorption, the column was washed with tap water (2 bed volumes) and adsorption cycle was restarted.

Figure 5.56 represents the results of two cycles of adsorption and desorption of mixed rare earths. While in the first cycle the column was run till $C_e = C_o$ before desorption, the second cycle was terminated at breakthrough ($C_e = 0.05\ C_o$) . It could be observed that there was no loss of capacity in the second cycle. The results clearly

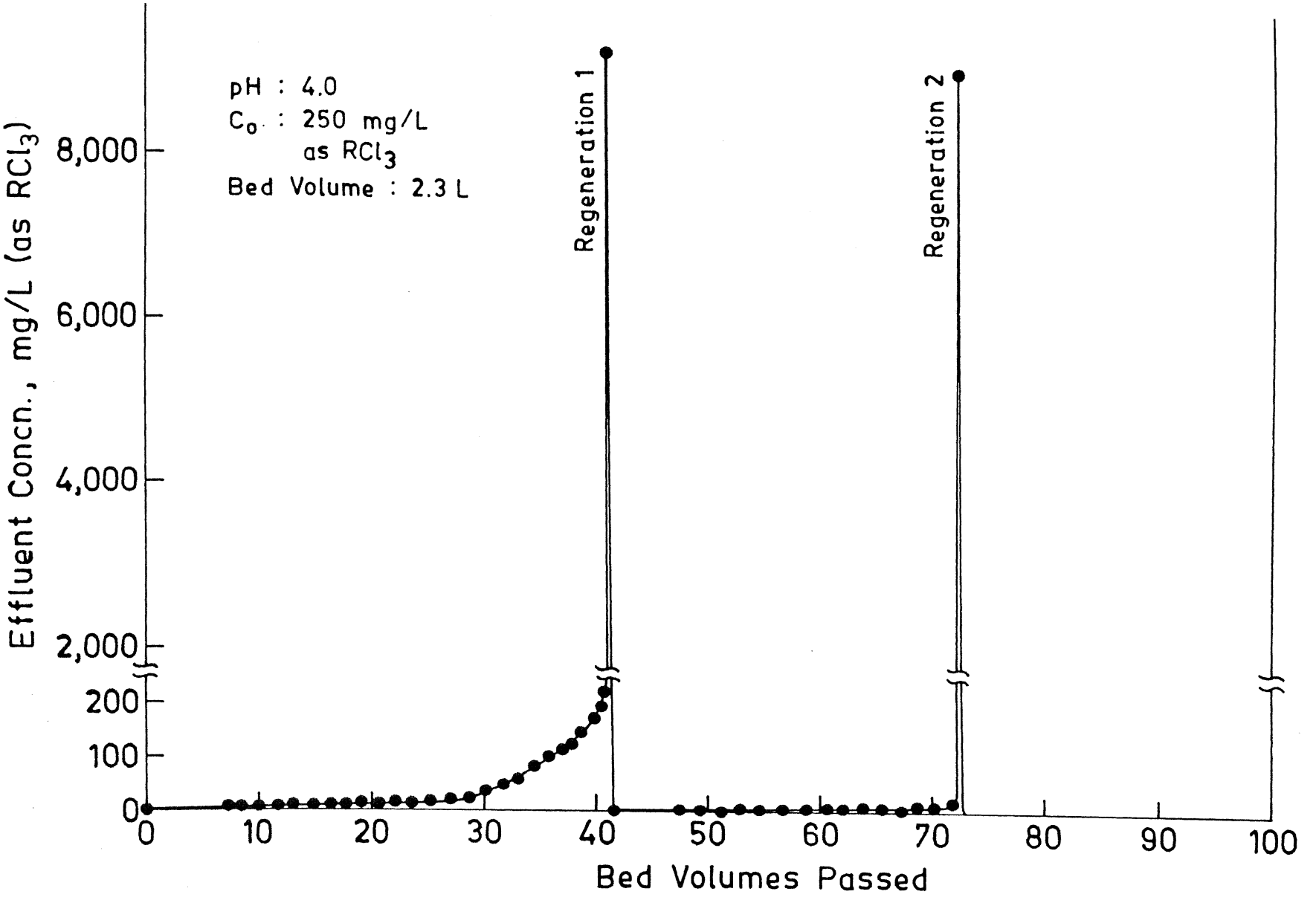


Fig. 5.56. Regeneration and Reuse of Biosorbent for Mixed Rare Earth.

indicate the excellent potential of *G. lucidum* for deployment in a packed bed reactor for uptake and concentration of rare earths.

5.9.5.4 Application of BDST Model.

Though the rational design of adsorption columns based on batch adsorption data and mass transfer coefficient, is not impossible, it is however desirable to carry out the column studies to obtain empirical relationships for the design. One of the widely used model is the Bed Depth Service Time (BDST) formulation (Benefield and others, 1982), which is a simplified form of Bohart Adams model based on surface reaction theory. Since the process of biosorption also is of chemical in nature, the use of this model may be appropriate.

According to Bohart-Adams model, which is based on the surface reaction theory, the equation to find out the service time of a column is given as

$$T = \frac{N_o X}{C_o V} - \ln [(C_o/C_b) - 1] \times 1/[C_o K] \qquad 5.38$$

Where

C_o = Initial concentration of sorbate
C_b = Desired concentration of sorbate at breakthrough
K = Rate constant
N_o = Adsorptive capacity of sorbent
X = Depth of column bed
V = Linear flow velocity of feed
T = Service time of column

The theoretical depth of adsorbent that is sufficient to prevent the effluent solute concentration from exceeding C_b at zero time is

called the critical bed depth (length of mass transfer zone) and can be calculated by equating T= o

$$X = \frac{V}{KN_o} \ln [(C_o/C_b)-1] \qquad 5.39$$

A series of 3 columns with internal diameter of 50mm. and varying heights 300, 600, and 1200 mm. was used for continuous flow studies. The influent was a mixed RCl_3 solution (Composition as given in Table.4.2) and buffered to a pH of 4.0. Since no effluent standards have been fixed for rare earths, the occurrence of breakthrough was taken when $C_e = 0.05C_o$. The flow rate maintained was 1.018 $m^3/m^2/h$, which fall within the flow rates in adsorption columns.

The time period for reaching breakthrough point for beds of different depths is plotted and is presented in Figure 5.57. The service time at breakthrough should be a linear function of bed depth if the flow rate and influent concentration are kept constant throughout the test. It can be observed from the figure that the linearity is maintained between the bed depth and the service time. This empirical plot can be used for the scale up of biosorption column. Design of adsorption columns can be done easily by finding out the bed depth or service time as the case may be from the plot for a specified set of influent characteristics. The values of constants obtained from the plot can be extrapolated for other influent concentrations and linear velocities as follows.

Simplified form of Bohart-Adams Model is

$$T = aX + b \qquad 5.40$$

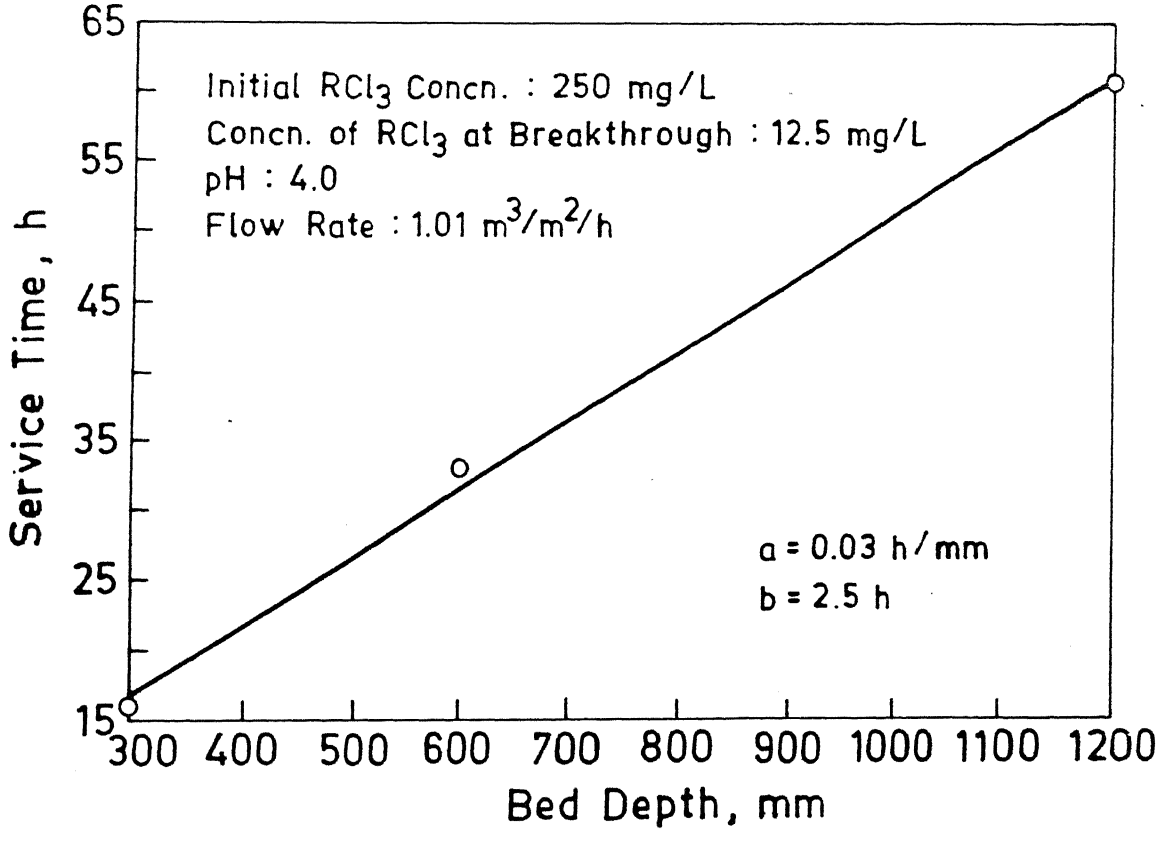


Fig. 5.57. Prediction of Constants for BDST Model.

where

$$a = \frac{N_o}{C_o V} \qquad 5.41$$

and

$$b = \ln[(C_o/C_b) - 1] \times 1/(C_o K) \qquad 5.42$$

These parameters can be evaluated from Figure 5.57. For a flow rate other than the one used in the development of constants, the equation can be modified by utilizing a new constant a′ such as

$$a' = (V/V') \times a \qquad 5.43$$

where V and V′ are the original and new flow rates. Similarly the BDST equation developed for one concentration can be modified so that it is applicable for another concentration as

$$a' = (C_o/C_1) \times a \qquad 5.44$$

and

$$b' = b \frac{(C_o)}{(C_1)} \times \frac{\ln[(C_1/C_F) - 1]}{\ln[(C_o/C_b) - 1]} \qquad 5.45$$

where C_o and C_1 are the old and new influent concentrations and C_b and C_F the old and new effluent concentrations.

The curve developed and the constants evaluated can thus be employed for the design of an adsorption columns over a range of feasible flow rates and concentrations.

5.10 BIOSORPTION FOR TREATMENT OF MONAZITE PROCESSING EFFLUENTS

Effluents emanating from rare earth processing industries represent one of the major sources of rare earth pollution. Because they are confined geographically to a few locations all over the world

in a small number of countries and mostly under the direct control of the respective Governments, pollution control from these industries has not yet been accorded the urgency it demands despite the recorded evidences of the toxicity of these effluents to human beings.

India is one of the major rare earth producing countries and deposits of rare earths are found on long stretches of beach along the western coast. The ore of rare earths in India is Monazite, which as described previously, is a mixture of rare earths and thorium phosphate. The presence of thorium makes processing of this ore even more hazardous and the disposal of resulting effluents more difficult (Sarat, 1992). It was therefore felt appropriate to apply the process of biosorption to remove the rare earths from the liquid effluents and recover it as a resource.

Monazite processing wastewater was simulated in the laboratory as per the composition given in Table 4.4.

The waste consisted of significant amount of anions (fluoride and phosphate), traces of heavy metals (zinc and lead) in addition to rare earths and thorium. The pH was maintained at 4.0 using acetate buffer. The simulated wastewater was passed through the column (50 mm ID) at a flow rate of 1.018 $m^3/m^2/sec$. As can be noticed from the breakthrough curve presented in Figure 5.58, the biosorbent *G. lucidum* could bring down the concentration of both rare earths and thorium to levels below detectability of the analytical methods employed (0.1 mg/L for thorium and 0.15 mg/L for RCl_3). The results highlight the potential of employing the biosorption by *G. lucidum* as a plausible alternative effluent treatment strategy in rare earth processing. The advantages of

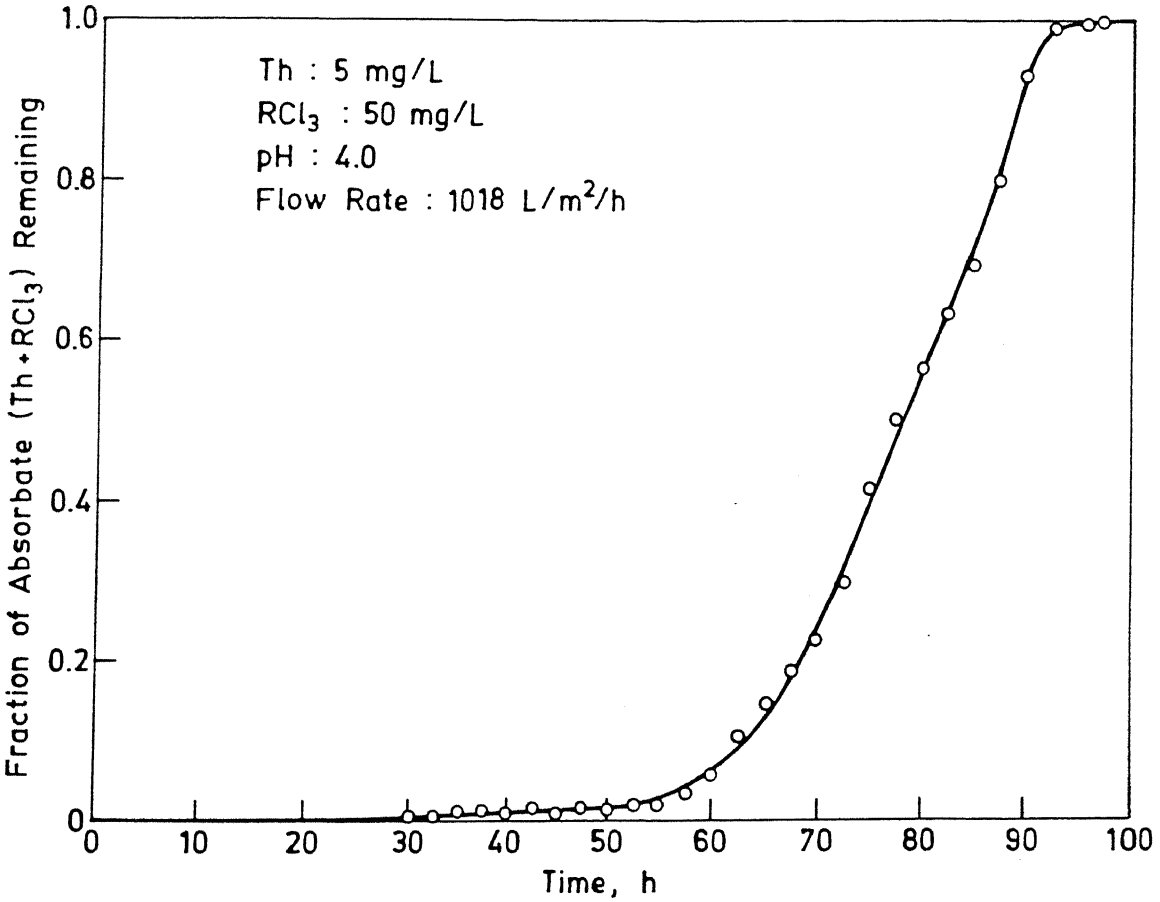


Fig. 5.58. Breakthrough Curve for Monazite Processing Effluent.

biosorption over conventional techniques of monazite industry effluent treatments are;

1. A single unit is necessary for removal of both thorium and rare earths. In the case of precipitation, it has to be a two step process for removal of Th and rare earths in two separate rectors followed by solid liquid separation facility.
2. The elements taken up could be desorbed using HCl and recycled back to the process stream. A modified version (Gschneidner, 1981) incorporating effluent lines and biosorbent reactor is presented in 5.59. Alternatively the biosorbent loaded with the toxic elements could be immobilized onto a cement concrete matrix for containment (similar to those employed in nuclear power plant effluents).

5.11 SEPARATION OF RARE EARTHS

Separation of rare earths are traditionally achieved by employing ion exchange resins. As the mode of uptake of metals by *G. lucidum* appears to be due to ion exchange, it was attempted to employ this biosorbent to achieve separation between rare earth elements. A mixture of neodymium and praseodymium was selected to evaluate the potential of *G. lucidum* to separate them.

The column was initially loaded with a mixture of neodymium and praseodymium at a flow rate of 1.018 m^3/m^2sec. The effluent was collected and UV-Vis spectra were taken as rare earth give characteristic signals in UV-Visible region. Further, UV-Vis spectra was utilized to monitor the progress of both adsorption and elution process.

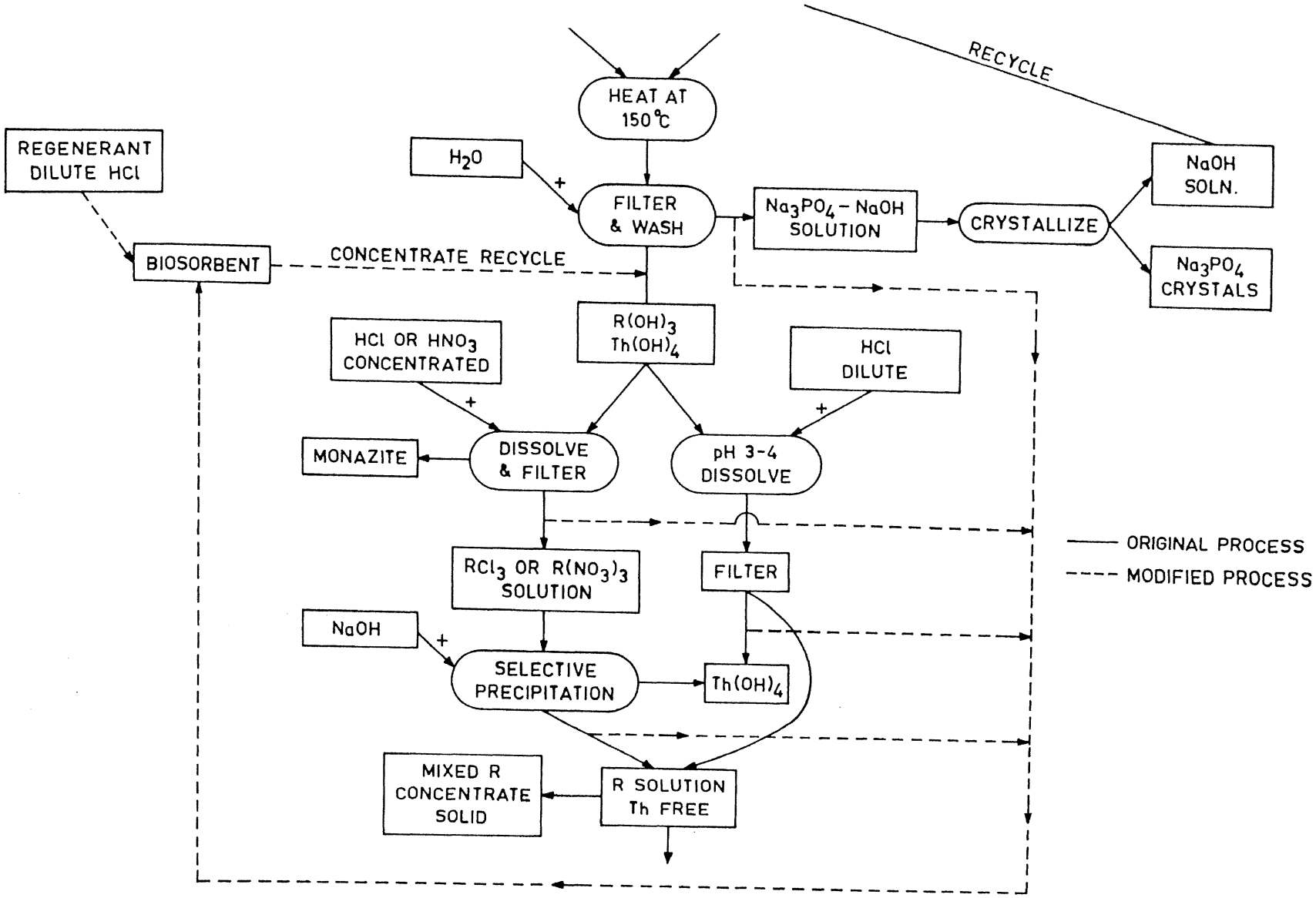


Fig. 5.59. Modifications for Recovery and Recycle of Rare Earths and Thorium from Monazite Processing.

Figure 5.60 (a) and (b) represents the UV-Vis spectra of the influent (Mixture of Pr and Nd) and effluent from the column during the adsorption cycle. The characteristic peaks of Pr (444 nm) and Nd (790, 742, 575) are clearly visible in the influent while only a very weak signal at 444 is present in the effluent.

The column was eluted with citric acid which is reported to be a suitable eluent. The UV-Vis spectra of effluent was scanned. Figure 5.61 (a) and (b) presented are two typical spectra obtained at 3 hours and 6 hours interval from the beginning of the elution cycle. The signals corresponding to neodymium were more pronounced than that of praseodymium indicating that a relative concentration of neodymium (separation from Pr) has been achieved.

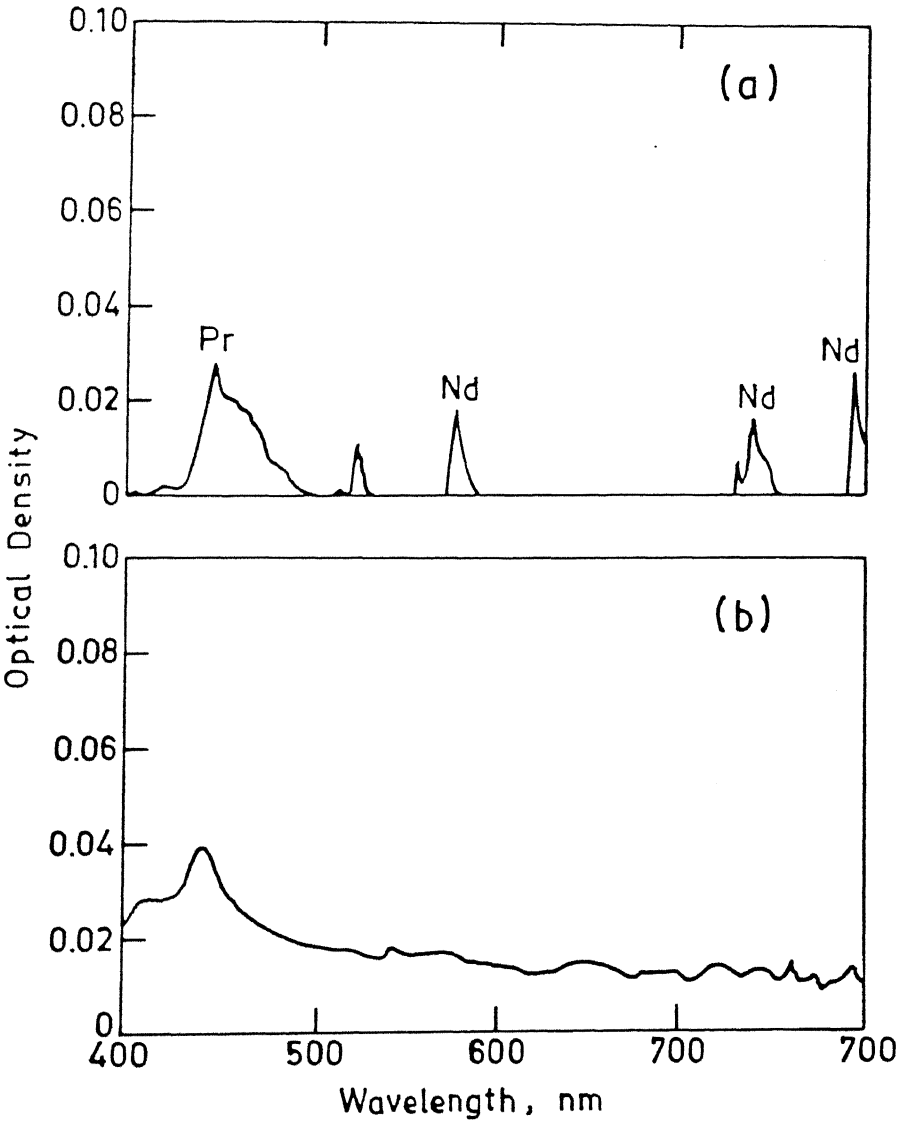


Fig. 5.60. Visible Spectra of a Mixture of Pr and Nd.
(a) Influent to Biosorbent Column
(b) Effluent from Biosorbet Column.

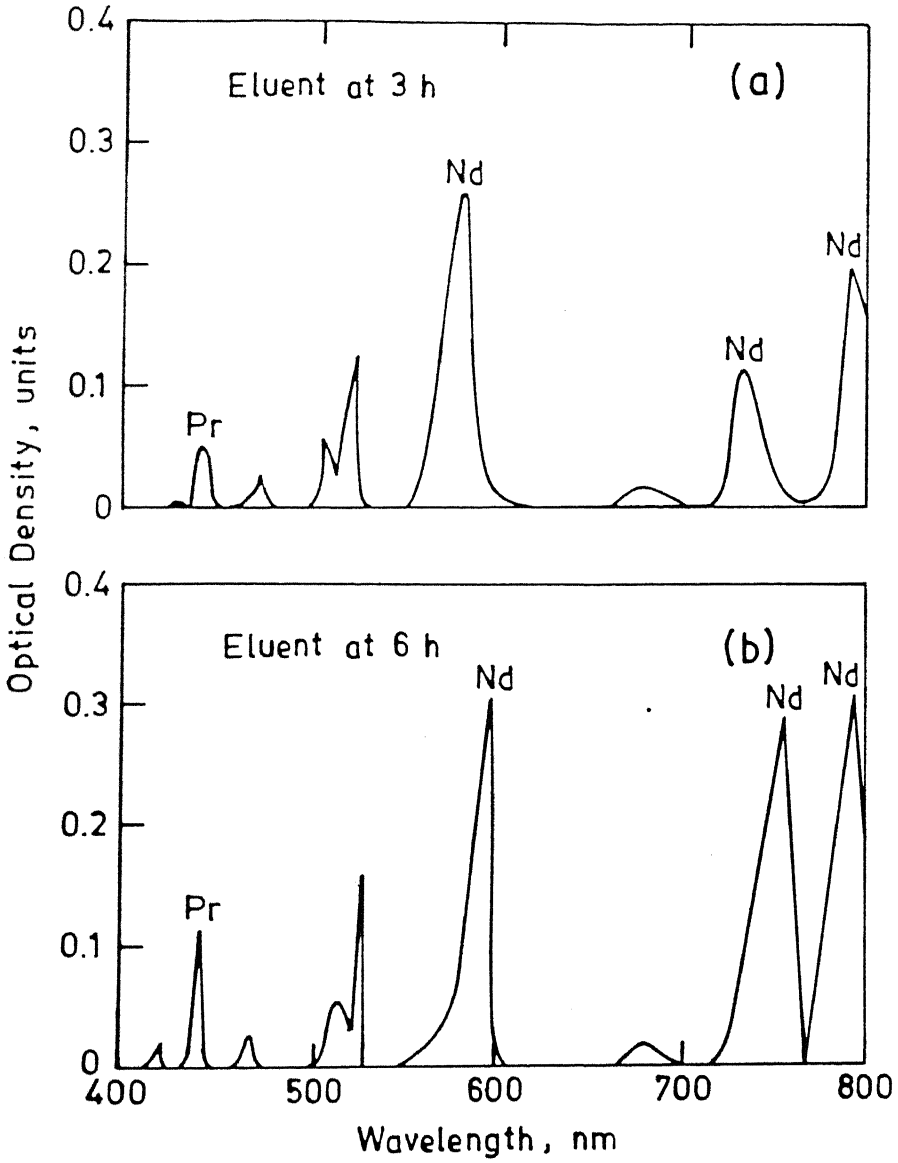


Fig. 5.61. Visible Spectra of Eluent from Biosorbent Column Loaded with Pr and Nd.
[Eluting Agent : 5 % Citric Acid, pH : 2.54, Temp. : 30 C, Flow Rate : 3 cm/sec]

6. SUMMARY AND CONCLUSIONS

The study indicated that a positive potential exists for the development of biosorbents with an excellent physico-chemical and metal uptake properties, using saphrophytic macrofungal fruiting bodies as the resource material. The study identified *Ganoderma lucidum* to be the most promising as a biosorbent of all the species screened. The sorbent properties could be modified by chemical treatments. A set of parameters relevant for effective comparison of biosorbents were developed to arrive at this conclusion. The uptake potential exhibited by the biosorbent was far in excess of that of activated carbon (Filtrasorb 400) and higher than many of the reported biosorbents with which field units have been developed. Studies on the mechanism of biosorption gave an insight into the site of interaction between metal and interacting groups. Ion exchange evolved as one of the major mechanisms of metal uptake and it could be established that the donor site is oxygen dominated. Kinetic studies revealed that the reaction followed reversible kinetics and the rate of reaction was high as compared to many other reported sorbents. Rate limiting step in biosorption of metals was identified as film diffusion.

Metal uptake by biosorbent *G. lucidum* was shown to be at the cell wall with structural polysaccharides probably being the main site of metal binding. The complexing ligand on the hyphal wall appeared to be rich in oxygen donor atoms and major fraction of the metal taken up was exchanged with calcium and hydrogen atoms.

In addition to copper, which was employed as the model metal, the biosorbent also took up other metals and preferential uptake was

observed with the oxygen seeking element lanthanum. Uptake of copper was affected by strong anionic ligands like cyanide and EDTA. The pH of the adsorbate solution also had significant impact on the metal uptake.

Application oriented studies were conducted using packed bed reactors. Packed bed reactors exhibited low head loss and nominal attrition losses at flow rates of an order of magnitude higher than those generally encountered in adsorption reactors. Mixed rare-earth effluents could be concentrated over 40 times in dilute mineral acids. It was also possible to bring down contaminant metal concentrations below toxic levels from simulated monazite processing industry effluents which contained high concentrations of fluorides and phosphates. This finding has substantial practical importance from both pollution control and resource recovery point of view. It was also possible to achieve separation between rare earths using a biosorbent column and suitable desorption medium.

The following conclusions may be drawn based on the results of the investigation

1. Fruiting bodies of saphrophytic macro fungi growing widely in humid tropical climates can be a potential source of raw material for the development of biosorbents.
2. *Ganoderma lucidum*, an wood rotting macrofungi exhibited a maximum uptake capacity of 0.383 mmol of copper/g among the various biosorbents investigated, which is comparable to those of other reported biosorbents and far in excess of activated carbon Filtrasorb 400.
3. Number of biosorbent derivatives prepared from *G. lucidum*, by various chemical treatments did not exhibit any enhancement in the

metal uptake. The chemical treatment could not also alter the microstructure of the biosorbent derivatives as evident by scanning electron microscopy.

4. A broad based comparison of relevant physico-chemical properties of all biosorbent derivatives along with metal uptake capacities indicated that *G. lucidum* does not require any chemical treatment to exhibit its maximum potential.

5. Transmission electron microscope studies indicated that the metal accumulation had occurred on the hyphal wall and not within the cytoplasmic pore.

6. EDAX of the biosorbent before and after copper uptake indicated that calcium ions originally present on the cell wall were exchanged for the adsorbate. This was confirmed by determination of calcium in the aqueous phase.

7. A detailed study of EPR spectra of the biosorbent before and after copper adsorption indicated the metal coordinating environment to be oxygen dominating.

8. Metal uptake is affected by the presence of anionic ligands but such an effect appears to be consequential in the case of only very strong ligands like EDTA and cyanide.

9. The kinetics of biosorption can be represented by a reversible model.

10. Film diffusion appeared to be the rate limiting step in the biosorption as indicated by both kinetics and interruption tests.

11. Head loss developed in a downflow packed bed adsorption reactor is negligible as well as the attrition losses (0.85 %).

12. Reactors employing *G. lucidum* could remove individual as well as mixed rare earths. The adsorbed metal could be concentrated over

12. Reactors employing *G. lucidum* could remove individual as well as mixed rare earths. The adsorbed metal could be concentrated over forty times using dilute mineral acids. The adsorbent could also be reused without loss of efficiency.

13. The Bed Depth Service Time (BDST) model appears to fit well the sorption data obatined from the continuous flow studies.

14. The waste water from monazite processing industry can be effectively treated by biosorption. The elements adsorbed could be recycled back to the process stream with dual advantages of achieving pollution control and resource recovery.

15. A mixture of praseodymium and neodymium salts were non selectively adsorbed by the *G. lucidum*. The elements could, however, be separated by selective elution with citric acid thus effecting the separation of otherwise difficult to separate rare earths.

7. SUGGESTIONS FOR FUTURE WORK

Based upon the findings of the present study the following suggestions may be made for future studies;

1. In light of the potential established for saphrophytic macrofungi, a comprehensive screening may be undertaken. This might lead to the identification of even better biosorbents.
2. The parameters suggested in this study for broad based evaluation of promising biosorbents could be supplemented and standardised.
3. Further evaluation of the coordinating macromolecule within biosorbent and its purification from natural sources and possible synthesis of biomimetic adsorbents.
4. Scaling up of biosorbent *G. lucidum* to pilot plant scale at monazite processing industry.
5. The potential of utilising this biosorbent for treatment of effluents of other metal processing industries to be investigated.

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